सत्यमेव जयते

INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI

I.A.R.I.6.
GIP NLK—H-3 I.A.R.I.—10-5-55—15,000

JOURNAL
OF
DAIRY SCIENCE

VOLUME XII

JANUARY, 1929, to NOVEMBER, 1929

1929
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

EDITORIAL BOARD

A. C. DAHLBERG, *Editor*
Geneva, N. Y.

ASSOCIATE EDITORS

C. H. ECKLES
St. Paul, Minn

E. G. HASTINGS
Madison, Wis.

O. F. HUNZIKER
Chicago, Ill.

C. W. LARSON
Chicago, Ill.

E. B. MEIGS
Baltimore, Md.

L. A. ROGERS
Washington, D. C.

H. A. RUEHE
Urbana, Ill.

PAUL F. SHARP
Ithaca, N. Y.

C. E. GRAY
San Francisco, Calif.

All correspondence regarding articles should be directed to A. C. Dahlberg, Editor, Geneva, N. Y.

All correspondence regarding advertising should be directed to the Publishers, The Williams & Wilkins Company, Baltimore, Md.

CONTENTS

JANUARY. No. 1

A Study of the Proteolytic Action of Specific Organisms and Groups of Organisms in Butter Made from Graded Cream. George Spitzer and E. H. Parfitt . . . 1

The Use of Citric Acid and Sodium Citrate in Starter Cultures. Hugh L. Templeton and H. H. Sommer . . . 21

A Study of the Factors Affecting the Growth of Dairy Heifers. Part II. C. B. Bender and J. W. Bartlett . . . 37

A Study of the Effectiveness of Sodium Hypochlorite in Sterilizing Creamery Equipment. E. L. Fouts . . . 51

The Relation between Weight and Fat Production of Guernsey Cattle. Charles W. Turner . . . 60

Studies on the Nutritive Value of Milk. I. The Production of Nutritional Anemia in Albino Rats through Exclusive Whole Milk Diets. W. E. Krauss . . . 74

The Relations of Temperature and Time of Forewarming of Milk to the Heat Stability of Its Evaporated Product. E. F. Deysher, B. H. Webb and George E. Holm . . . 80

The Nutritional Value of Copper in Powdered Whole Milk. R. W. Titus and J. S. Hughes . . . 90

MARCH. No. 2

The Application of Steam for Heating and Sterilizing Dairy Equipment. A. W. Farrall . . . 95

Proteolysis by *Streptococcus lactis*. L. T. Anderegg and B. W. Hammer . . . 114

The Effects of Certain Salts on the Physical Properties of Ice Cream Mixes. J. C Hening and A. C Dahlberg . . . 129

Metals in Dairy Equipment. Metallic Corrosion in Milk Products and Its Effect on Flavor. O F. Hunziker, W. A. Cordes, and B. H. Nissen . . . 140

The Bacterial Count of Ice Cream Held at Freezing Temperatures. John Weinzirl and Alice E. Gerdeman . . . 182

MAY. No. 3

Arthur Christopher Baer, 1884–1929 . . . 191

The Use of Egg Yolk in Ice Cream. W. H. Martin and W. J. Caulfield . . . 193

The Ability of *Escherichia coli* and *Serratia marcescens* to Survive 62.8°C., for Thirty Minutes in Milk. Fred W. Tanner and Mary F. Windsor . . . 202

Some Factors Affecting the Fat Clumping Produced in Milk and Cream Mixtures When Homogenized. F. J. Doan . . . 211

The Antirachitic Value of Irradiated Ice Cream. Walter C. Russell, Forrest C. Button, and Orme J. Kahlenberg . . . 231

Studies on the Nutritive Value of Milk. W. E. Krauss . . . 242

Metals in Dairy Equipment. O. F. Hunziker, W. A. Cordes, and B. H. Nissen.......... 252
A Device for Reducing an Ice Cream Mix to Its Basic Viscosity. Randall Whitaker.......... 285

JULY. No. 4

The Relation of the Hydrogen-ion Concentration to the Texture of Emmenthal or Swiss Cheese. Paul D. Watson.......... 289
The Compartive Accuracy of the Direct Microscopic and Agar Plate Methods in Determining Numbers of Bacteria in Milk. James D. Brew..... 304
The Use of Dehydrated Egg Products in the Manufacture of Ice Cream. William S. Mueller and Forrest C. Button.......... 320
A Suggested Simplification of the Present System of Official Testing with the Dairy Breeds of Cattle. R. B. Becker and P. C. McGilliard.......... 337
The Mold and Yeast Count as an Index of the Keeping Quality of Butter. H. Macy and H. B. Richie.......... 351

SEPTEMBER. No. 5

A Comparison of the Bromthymol Blue Milk Test and the Methylene Blue Reduction Test for Determining Quality of Milk. E. D. Devereux..... 367
A Survey of Present Methods of Grading Milk at Condenseries, Evaporated and Dry Milk Plants.......... 374
A Color Defect of Process Cheese Walter V. Price.......... 377
Copper in Dairy Products and Its Solution under Various Conditions. II. J. Miscall, G. W. Cavanaugh and P. P. Carodemos.......... 379
An Unappreciated but Important Factor in the Pasteurization of Milk. J. M. Sherman, C. N. Stark and Pauline Stark.......... 385
Further Studies of the Rôle of Vitamin C in the Nutrition of Calves. L. M. Thurston, L. S. Palmer and C. H.Eckles.......... 394
The Solubility of Calcium Phosphate in Fresh Milk. E. O. Whittier.......... 405
Effect of Cottonseed Meal and Linseed Oil Meal on the Consistency of Feces of Dairy Cattle. C. F. Huffman and L. A. Moore.......... 410
Book Reviews.......... 419

NOVEMBER. No. 6

Contribution to the Knowledge of the Taste of Milk C. L. Roadhouse and G. A. Koestler.......... 421
Studies on the Nutritive Value of Milk. III. The supplementary Value of Various Constituents of Synthetic Basal Rations W. E. Krauss....... 438
The Physiologcal Effect of Rations Restricted Principally or Solely to the Alfalfa Plant. I. The Calcium, Phosphorus and Nitrogen Metabolism of Dairy Cattle. J. R. Haag, J. S. Jones, I. R. Jones and P. M. Brandt... 445
The Calculation of Absolute Viscosity from Data Secured by Means of the Torsion Pendulum Viscosimeter. Randall Whitaker and B. L. Herrington.......... 456
Effect of Heat (Oestrum) on Butterfat Percentage and Milk Yield. Lynn Copeland.......... 464

The Use of the Mojonnier Milk Tester for the Routine Determination of Vanillin with Preliminary Discussion of the Towt Lead Number. L. V. Towt.......... 469
A Comparison of the Modified Babcock and the Mojonnier Methods for Butterfat in Ice Cream. E. S. Chase and F. G. King.......... 473
Variations in the Milk from Different Quarters of the Same Udder: Their Significance in Studies of Coagulability. Anne G. Benton.......... 481
Seasonal Variations in the Feezing Point of Milk. J. H. Buchanan and O. E. Lowman.......... 484
The Ovarian Hormone Content of Pregnant Cow's Urine. C. W. Nibler and Chas. W. Turner.......... 491
Proceedings of the Annual Meeting of the American Dairy Science Association.......... 507
Index.......... 513

A STUDY OF THE PROTEOLYTIC ACTION OF SPECIFIC ORGANISMS AND GROUPS OF ORGANISMS IN BUTTER MADE FROM GRADED CREAM*

GEORGE SPITZER AND E. H. PARFITT

Purdue University, Department of Dairy Husbandry, Lafayette, Indiana

Much work has been done in the past concerning the factors involved in the deterioration of butter when held in storage. The earlier work had been directed mainly to the butter from the time it left the churn until the expiration of the storage period. Abnormal flavors which developed during storage, directed the attention of the investigators to the study of certain types of organisms or groups of organisms present in the butter which might be the cause of producing these abnormal flavors.

Of the earlier investigators, Otto Rahn and co-workers (1) made an exhaustive study of the keeping qualities of butter. The butter for this study was taken from tubs as found in creameries; and bacteriological examinations were made to determine the kind and number of organisms. These butters were placed in cold storage and chemical and bacteriological analysis made when fresh and at regular intervals during the storage period. It was shown that there was an increase in amide (amino) nitrogen during storage, and the poorer the quality of butter at the end of the storage period the greater was the increase of amide nitrogen. There was apparently no correlation of the activity of the organisms and the depreciation of the quality of the butter.

Fisher and Gruenert (2), although their work related to the keeping qualities of butter when antiseptics were added, found that the quality of the butter deteriorated in proportion to the increase of amide nitrogen. These butters were stored in a cellar of rather uniform temperature. The progress of protein hydrolysis was rapid but the final results were consistent and comparable.

---

* Received for publication July 5, 1928.

In 1909 Rogers and Gray (3) studied the influence of acidity of cream and the flavors of butters. Very little was known at that time concerning the changes taking place in the chemical composition of butter and the relation of these changes to the flavor and factors controlling them. In 1913 Rogers, Berg and coworkers (4) investigated the factors influencing the changes in flavor in butter during storage. These experimental studies involved the influence of the acidity of the cream, the presence of metals and bacteria, and the chemical changes of the proteins during storage. The experimental butters were kept in cold storage at −7°C. and analysis made when fresh and at periods of 200 to 300 days. The proteolysis progressed in all stored butters. These workers found that bacterial enzymes in milk held in cold storage containing 18 per cent of salt gave evidence of proteolysis. The increase in protein hydrolysis was related to the depreciation of the butter.

The results of the foregoing investigations, which were limited to the butter made from the better grades of cream, suggested the advisability of studying the keeping qualities of butter made from sour farm-skimmed cream. In 1911–1916, O. F. Hunziker, formerly head of the Dairy Department, Purdue University, planned and outlined an extensive investigation in the pasteurization of sour farm-skimmed cream for butter making, and a study of the keeping quality of this butter during storage. The results were compared with the butter made from the same cream not pasteurized. These investigations extended over a period of five years, and the results of these investigations have been reported by Hunziker and Spitzer (5). The experimental work in these investigations included the bacterial study of the cream and the butter made from this cream, both pasteurized and raw cream. A chemical study of the butter and protein hydrolysis was made when the butter was fresh and at intervals during storage. During the five-month period that the butter was held in cold storage, there was a gradual increase in the soluble proteins. This increase was greater in the butter made from raw sour cream than in the butter made from pasteurized sour cream. The increase of the soluble proteins was closely related to the quality of the butter as shown by the score, when the butter went into storage and at the end of the storage period.

In 1923 Ferris (6) made an extensive investigation on the development of soluble proteins in cream and butter during storage. This work was a decided advance in the study of protein hydrolysis. He made determinations of the soluble nitrogen compounds of the cream, and the corresponding butters when held in cold storage and in an ice box. The butters kept in cold storage showed an average increase in soluble nitrogen compounds of 1.2 per cent and the score decreased 2.7 points. These butters were made from gathered cream of only fair quality.

OBJECT OF THIS INVESTIGATION

The following investigations were outlined to study the cause of the depreciation of butter during storage, and to show the relations of what seemed to be the predominating factors influencing the flavor and general quality of the butter when held in storage.

1. The effect of inoculating pasteurized graded cream with proteolytic type bacteria.
2. The study of commercial butter as found on the Indiana market and as manufactured by Indiana Creameries.
3. The study of storage butter exhibited at the National Dairy Show, 1926, representing different sections of the American butter industry.

THE EFFECT OF INOCULATING PASTEURIZED GRADED CREAM WITH SPECIFIC ORGANISMS ON THE QUALITY OF THE RESULTING BUTTER WHEN HELD IN STORAGE

In the first part of our investigations, graded cream was used and inoculated with the following specific organisms: *B. mycoides*, *B. liquifaciens*, *B. subtilis*, *B. mesentericus*, *B. amylobacter*, *B. butyricus*, *B. ichthyosmius*, and *B. proteus vulgaris*.

The cream used in these experiments was No. 1 grade of gathered cream, the acid content varying from 0.09 to 0.36 per cent, while the fat content ranged from 33 to 45 per cent.

The cream was pasteurized in an open steam-jacketed vat at temperatures varying from 148° to 155°F. and held for twenty-minute periods. It was then cooled to a temperature of 80°F., and divided into three equal portions; one portion served as a control and each of the other two were inoculated with 1000 cc. of a twenty-four-hour broth culture of the specific organisms. Five cubic centimeters of a twenty-four-hour culture were used to inoculate the broth. All three portions were held at room temperature for sixteen to eighteen hours, then cooled to about 55°F., and churned. One-half of the butter from each portion of cream was salted, and five one-pound samples were taken from each of the finished butters. The butter was examined for quality when fresh and at intervals of thirty, sixty, ninety, and one

TABLE 1

Increases in soluble proteins as nitrogen not precipitated by phosphotungstic acid and nitrogen as peptones, and the description of the quality of the corresponding butters after storage of 180 days

INOCULATIONS	NOT SALTED			SALTED		
	Percentage increase of nitrogen not precipitated by phosphotungstic acid	Percentage increase in peptones	Description of flavor of butter	Percentage increase of nitrogen not precipitated by phosphotungstic acid	Percentage increase in peptones	Description of flavor of butter
Control	1.07	1.35	Lardy, flat	0.90	0.81	Lardy, flat
B. mycoides	1.46	3.23	Oily, flat, stale	2.05	2.19	Clean, flat
B. liquifaciens	1.28	3.11	Flat	1.69	3.27	Clean, flat
Control	1.32	1.96	Flat	1.78	3.05	Flat, greasy
B. subtilis	3.47	2.24	Flat, greasy	4.33	1.77	Flat, greasy
B. mesentericus	1.43	2.18	Flat, greasy	2.14	2.10	Flat, oily
Control	2.30	2.07	Flat, oily	2.38	1.94	Flat, greasy
B. amylobacter	2.55	1.51	Musty, flat	1.82	1.89	Musty, flat
B. butyricus	0.71	2.31	Flat, oily	0.60	3.05	Flat, oily
Control	2.18	4.36	Acid	1.82	4.30	Stale, oily
B. ichthyosmius	3.02	6.34	Unclean, cheesy	2.35	8.09	Stale, bitter
B. proteus vulgaris	2.16	7.76	Stale, unclean	2.66	6.33	Cheesy, stale

hundred eighty days. All samples were held in cold storage during this period at 0° to 4°C. (32° to 39°F.).

In the chemical analysis of the protein hydrolysis, we have used the methods of Spitzer, and co-workers (7). The chemical analysis consisted in determining the soluble nitrogen compounds not precipitated by phosphotungstic acid and nitrogen compounds not precipitated by saturated solution of zinc sulphate.

It will be noted from table 1 that protein hydrolysis proceeded in all samples of butter during storage. In discussing the results we must bear in mind that pasteurization does not destroy all organisms in the cream which will develop during ripening. In general, all inoculated butter showed a greater decrease in quality than the controls or the not inoculated. The most pronounced changes in quality and protein hydrolysis were produced by the two proteolytic organisms, *B. ichthyosmius* and *B. proteus vulgaris.*

TABLE 2

Average increase in nitrogen compounds not precipitated by phosphotungstic acid and in peptones in the uninoculated and inoculated butters after 180 days of cold storage

	NOT SALTED		SALTED	
	Per cent of nitrogen not precipitated by phosphotungstic acid	Per cent peptones	Per cent of nitrogen not precipitated by phosphotungstic acid	Per cent peptones
Not inoculated	1.72	2 44	1.97	2 53
Inoculated	2.01	3.77	2.20	3 62

The depreciation in quality was quite consistent with the chemical changes in proteins of the butter, there being an exception in protein cleavage when cream was inoculated with *B. butyricus.* The nitrogen compounds were much less for *B. butyricus* than the control, while the peptones were consistent. This action may be accounted for as previously stated. A brief summary of results in table 2 clearly show the effect in general on the changes in the proteins made from the inoculated cream when compared with the control.

From this summary and the average cleavage products for butter made from cream not inoculated and from butter made from inoculated cream there is an increase of soluble nitrogen in the inoculated butter apparently in proportion to the decrease in quality.

THE RELATION OF PROTEOLYSIS, HYDROGEN ION CONCENTRATION, AND KEEPING QUALITY OF INDIANA BUTTER

A study was made of commercial butters as found on the Indiana market, and manufactured by Indiana creameries representing every part of the state. Samples of butter were collected during the months of July, September, and November, 1926; and January and March, 1927. The samples consisted of one-pound cartoned packages of print butter and represented 40 different brands of Indiana butter. The butters were purchased from the retailer at grocery stores and meat markets in many Indiana cities. The samples were then sent by mail, special delivery, to the Dairy Department of Purdue University. Upon receipt of the butter at the laboratory, it was held at a temperature of 10°C. for about one day, until it was in proper physical condition to be sampled and scored.

A total of 241 samples was collected and examined in this manner. The samples which scored 90 points or better were selected for the study of the proteolytic decomposition during a storage period of 180 days at a temperature of 10° to 0°C. Thirty-four samples were thus selected. Each of these samples was divided into equal parts. One part was used for immediate analysis, the other part was stored in Mason jars. At the end of the storage period, the butter was scored and analyzed again. In this manner, comparative scores and analyses were secured for the butters at the beginning and at the end of the storage period.

The analytical work in this series consisted in determining the peptones and the soluble nitrogen compounds not precipitated by phosphotungstic acid.

In discussing the depreciation of butter from the point of view of protein hydrolysis, we have found that there is an unmistakable relation between the peptones and nitrogen compounds not precipitated by phosphotungstic acid, and the depreciation of the butter during storage.

In table 3 are tabulated the average increases in the peptones and the nitrogen not precipitated by phosphotungstic acid, and scores for each group of butter. These groups are in order of the season of the year in which they were collected. There was some increase in the soluble nitrogen compounds in November, January, and March. The butters which had the best keeping quality, were those of group C which were collected in November and those which decreased in score the most, were butters in group A collected in July, and butters in group E collected in March.

TABLE 3

The average increase in nitrogen compounds not precipitated by phosphotungstic acid and in the peptones, the average score of the butter when fresh and at the end of the storage period of 180 days

LOT	PERCENTAGE INCREASE IN NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	PERCENTAGE INCREASE IN PEPTONES	SCORE WHEN FRESH	SCORE AT END OF STORAGE	LOSS IN SCORE	MONTH WHEN COLLECTED
A	1.79	1.10	90	88	2 0	July, 1925
B	1.57	0 95	90 3	88 7	1 6	September, 1925
C	2.08	2.25	90 5	89 3	1 2	November, 1925
D	2 37	2 14	90 4	88 5	1 9	January, 1926
E	2 40	2.51	90 1	87.1	3 0	March, 1926

In recent years considerable attention has been given to the hydrogen-ion concentration of dairy products. This is also true in the bacteriological study of the growth, development and metabolism of organisms in different media. In biochemical study the control of hydrogen-ion concentration has become an important and decisive factor in regulating and controlling chemical reactions.

We deemed it worth while to determine the hydrogen-ion concentration colorimetrically of those experimental butters. The method used to determine the pH of these butters was the dilution of 1:10 in neutral water, then determining the pH of the water. The determinations of the pH value were made when

TABLE 4

The pH value, increase in nitrogen not precipitated by phosphotungstic acid and decrease in score

NUMBER	pH	N NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	DECREASE IN SCORE
Group pH 4.5 to 5			
		per cent	*per cent*
A 30	4.8	0.50	2.0
A 40	4.6	1.80	1.0
Average		1.15	1.5
Group pH 5 to 6			
B 33	5.8	1.79	1.0
C 38	5.8	1.70	2.0
C 45	5.9	2.02	1.0
C 47	5.8	0.93	0.0
D 15	5.8	2.06	1 5
Average		1.46	1 0
Group pH 6 to 7			
A 28	6.0	3.08	3.0
B 4	6.0	2.05	1.5
B 12	6.9	1.01	3.5
C 10	6.6	1.80	0.5
C 35	6.0	1.50	1.5
D 26	6.0	2.44	2.5
E 8	6.27	4.30	4 0
E 18	6.4	1.80	2 0
Average		2.25	2.3
Group pH 7 plus			
B 5	7.3	1.94	0.5
B 16	7.2	2.05	1.5
C 3	7.6	2.29	2.0
C 5	7.6	1.90	1.0
C 8	7.0	4.29	2.0
C 13	7.4	1.25	1.0
C 34	7.2	3.03	1.0
C 37	7.3	3.14	0.5
D 27	7.6	0.72	2.5
D 24	7.6	2.78	1.5
E 3	7.2	2.22	4.0
E 7	7.4	1.70	2.5
E 13	7.0	1.96	3.5
Average		2.25	1.80

butter was fresh before being placed in storage. Table 4 gives the pH value for 30 samples of the butter, also the increase of nitrogen compounds not precipitated by phosphotungstic acid and the decrease in score. The division into groups was made on the basis of the pH value, those butters ranging from 4.5 to 5, 5 to 6, 6 to 7, and 7 plus.

Table 4 shows some interesting and valuable information based on a limited number of samples of butter. When butter had a pH of 4.5 to 5 there was a decrease in score of 1.5 points, also an increase in nitrogen not precipitated by phosphotungstic acid. Having but two samples of butter in this group no important conclusion can be drawn. When the butters had a pH of 5 to 6 the butter decreased in the average of the score 1 point, and the nitrogen compounds increased, 1.46 per cent. Butters having a pH of 6 to 7 decreased in the average score 2.3 points and increased in nitrogen not precipitated by phosphotungstic acid 2.25 per cent and those butters having a pH 7 and higher, decreased in score 1.80 points, and increased in nitrogen not precipitated by phosphotungstic acid 2.25 per cent.

There are some well established facts to account for the above results. It was shown in our previous work (7) on the study of the enzymes of specific organisms upon the proteins of milk, that the proteolytic changes are due to the action of enzymes. These enzymes are produced by proteolytic organisms and these enzymes appear in the butter and continue their action during storage. Many enzymes are derived from various microörganisms, but we are concerned here principally with the proteolytic types. There are two important groups of proteolytic enzymes, the peptic and the tryptic. The survey of the literature indicates that the chemical behavior common to enzymes involves the increase or decrease of the activity by the variation of the pH value of the substrate. The optimum pH has been determined for a number of enzymes. Every enzyme has a zone of a greater or lesser activity. The pH value for the optimum activity of the peptic enzymes lies between 1.5 to 4.0, depending on the source of the enzyme, while the tryptic enzymes have a much higher pH value for greatest activity ranging from 6 to 8. Making use of these principles, we can in a great measure explain or account for the changes in the protein hydrolysis in the butters as grouped in table 4. We must remember however, that the enzyme action progresses slightly on either side of the zone of optimum activity. Butters having a pH of 4.5 to 5.0, the pH value are not in the range of the tryptic enzyme action, but would be more in range of the peptic action, and as the pH value increases we would expect and do have a greater tryptic action on the proteins. There is a significant difference in the products formed by peptic and by tryptic enzymes. The peptic enzymes do not hydrolyze proteins beyond the peptone stage, at least not to a very large extent, while the tryptic enzymes produce in addition to peptones, amino acids or the simplest units of the protein. The nitrogen not precipitated by phosphotungstic acid represents the great portion of the amino acids formed during hydrolysis. The above principles concerning enzyme action are to a reasonable extent corroborated in table 4.

THE RATE OF PROTEOLYSIS AND DECREASE IN FLAVOR OF STORAGE BUTTER AT THE 1926 DAIRY SHOW

One of the classes for butter at the National Dairy Show is known as the "Storage Butter Class." The butters came from all the leading dairy sections of the United States and Canada, and gave a very representative group as to methods of manufacture and conditions under which cream from buttermaking is produced. At the Dairy Show held in Detroit in 1926 this class was scored when entering storage in July and upon leaving storage in October. In this class were 183 packages of butter, the packages varying in size from 10 pound boxes to 86 pound tubs. The object of this scoring was to determine which butter possessed the best keeping quality during the storage period.

Samples for chemical analysis were obtained from one-half of the package at the first scoring and the other half at the second scoring. Three plugs of butter were removed from the package using a thoroughly wiped 12 inch butter trier, placed in glass topped Mason jars, and kept in cold storage at 0° to 5°C. till ready for analysis.

In this group only samples that scored 90 points or more when entering storage were analysed. The samples of butter were analysed when fresh and at the end of the storage period of ninety days. The following fractions of protein hydrolysis were determined: nitrogen compounds not precipitated by phosphotungstic acid; nitrogen compounds not precipitated by saturated

TABLE 5

The increase in nitrogen not precipitated by phosphotungstic acid, and in peptones during storage of the National Dairy Show butters and the score of the butter when fresh and after storage

NUMBER	INCREASE IN NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	INCREASE IN NITROGEN AS PEPTONES	SCORE WHEN FRESH	SCORE AFTER STORAGE
	per cent	*per cent*		
3	0.47	1.26	93.5	93.5
5	0.64	0.13	92	90
9	0.99	−0.40	90	90
12	0.53	0.17	89	91
24	1.18	0.94	94.7	93.5
36	1.55	0.42	93.5	90.5
39	0.65	0.06	91.5	90
58	1.13	0.03	91	91.5
64	1.31	1.09	93.5	93
68	0.24	0.19	94	93.5
79	0.30	1.27	93	92
86	0.01	0.17	92	91
90	0.68	1.89	92.5	93
101	1.82	3.89	94	93
107	0.89	3.80	93.5	92
114	0.23	3.99	93.5	93
121	1.42	1.16	94	93.5
132	0.92	4.31	93.5	93
145	0.88	5.69	93	93
147	0.82	4.14	90	89
150	0.44	1.37	90.5	90.5
161	−0.16	1.79	92.5	92.5
165	1.52	2.06	94	92
172	1.03	−0.12	93.5	94.2
176	0.12	3.38	93	92.5
183	0.22	0.53	91	92
184	−0.15	0.28	92.5	94
185	1.15	−0.10	91	90

solution of zinc sulphate; and the peptones which represented the difference between the two precipitants.

A study of table 5 shows that there were 28 samples of butter which during storage increased less than 1 per cent in nitrogen that was not precipitated by phosphotungstic acid, and that these butters lost but an average of 0.22 point in score. Where the loss of nitrogen not precipitated by phosphotungstic acid was more than one per cent of which there were 28 butters, the average loss in score was 1.07 points.

TABLE 6

A summary, compiled from table 5, showing relation of change in score and in nitrogen not precipitated by phosphotungstic acid

CHANGE IN SCORE	NUMBER OF BUTTERS	INCREASE OF NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID
		per cent
Increased	6	0.62
Loss of 0.5 point	6	0.71
Loss of 1.0 point	5	0.82
Loss of 1.5 points or more	6	0.97

TABLE 7

Summary, compiled from table 5, showing change in score and in peptones

CHANGE IN SCORE	NUMBER OF BUTTERS	INCREASE OF PEPTONES
		per cent
Increase	6	0 48
No loss or gain	5	1.98
Loss of 0.5 point	6	2.35
Loss of 1.0 point	5	2 17
Loss of more than 1.0 point	6	1.24

The relationship of nitrogen not precipitated by phosphotungstic acid to the loss in score of butter is indicated in table 6 which is a summary table of table 5.

A summary table 7 shows the relation of the increase in peptones to the gain or loss in score of butter during storage.

THE MICROBIOLOGICAL ANALYSIS OF THE STORAGE BUTTER OF THE 1926 DAIRY SHOW

Samples of butter were taken from one-half the package in June and from the other half in October. Sterile butter triers were used and the samples placed in sterile glass jars and held at 0° to 4°C. until analyzed.

Method of analysis

The samples of butter were placed in a 40°C. water bath and allowed to melt until they were of the consistency of heavy cream. Five grams of this melted butter were weighed into 45 grams of sterile water which had been previously warmed to 37° to 40°C. Each sample was then agitated and dilutions were prepared on a volumetric basis.

The total bacterial count was obtained by plating, using skim milk powder agar as described by Ayers and Mudge (8). Incubation was for five days at 20°C. followed by one day at 37°C. The acid type bacteria were obtained by plating, using skim milk powder agar as mentioned above, but to this medium was added 1 per cent of lactose and 0.0016 per cent of brom cresol purple. Incubation of these plates was the same as for the total count. Acid organisms were those which caused the change of the indicator from purple to yellow. These plates and counts also served as a check for the total count plates. The non-acid forming group of bacteria was determined by subtracting the acid forming colonies on the plates from the total bacterial count.

A group of bacteria termed proteolytic was determined by the liquefication of gelatine, even though we recognized that this characteristic of bacteria might not signify that they attacked casein. The liquefication of gelatine was taken as an index and used because of its convenience. The count was made after incubation of fifteen days at 20°C.

Yeasts and molds

This group was determined by growth upon acidulated whey agar after five days incubation at 25°C.

The data were tabulated to determine if growth of the microorganisms took place in the butter during the cold storage period of approximately three months. The results are found in table 8.

From table 8 it is shown that there was a slightly greater number of butters that showed increase in numbers of total, non-acid type, and proteolytic type bacteria while there was a decrease in the majority of butters in the acid types, yeasts and molds.

TABLE 8

Percentage of butters showing changes in numbers of microörganisms during storage

GROWTH	TOTAL BACTERIA	ACID TYPE BACTERIA	NON-ACID TYPE BACTERIA	PROTEOLYTIC	YEASTS	MOLDS
Increased	52 45	41.95	46 65	44 00	21 90	23 20
Decreased	42 95	52 45	44 55	40 70	54.25	50 25
No change	4 60	5 60	8 80	15 30	13 85	26 55

TABLE 9

The change in score of 69 butters of the National Dairy Show of 1926 which upon entering storage contained more than 10,000 gelatine liquefying type bacteria per gram

CHANGE IN SCORE	NUMBER OF BUTTERS	CHANGE IN NUMBERS OF GELATINE LIQUEFYING BACTERIA			
		Increased		Decreased	
		Number	Per cent	Number	Per cent
Increase	17	7	41 17	10	58 83
No change	16	4	25 00	12	75.00
Loss of 0.5 point	21	17	80.92	4	19.00
Loss of 1 point	15	15	100.00	0	0.00

Tabulating the effect of increase or decrease of proteolytic type organisms upon the changes in score of the butter during storage it was found upon first analysis for proteolytic bacteria that there were 69 butters which showed 10,000 or more proteolytic bacteria per gram. Upon leaving storage in October, 15 of these 69 butters had lost one point in score and had all increased in proteolytic bacteria during storage. Twenty-five per cent of those butters which showed no change in score increased in proteolytic bacteria, while 75 per cent decreased in proteolytic bacteria during the storage period. The results are tabulated in table 9.

The activity of those bacteria which liquefy gelatine in the butter reflects upon the change in score during storage, for as the score diminished the growth of these bacteria increased, especially is this true for the butters that decreased in score during storage.

The salt concentration and its relationship to the growth of microörganisms in the butter was tabulated. It was found that salt inhibited the growth of microörganisms since the number of butters showing increases in bacterial growth during storage decreased as the salt concentration in the brine increased.

TABLE 10

The effect of salt on per cent of butters increasing in numbers of microörganisms during storage

PER CENT OF SALT IN BRINE	TOTAL BACTERIA	ACID	NON-ACID	PROTEOLYTIC	YEASTS	MOLDS
0–5	70.00	50.00	70.00	60.00	40.00	40.00
5.1–10.0	67.01	51.25	59.13	51.25	48.62	30.22
10.1–15.0	48.55	41.44	37.12	41.41	21.42	14.28
15.1–20.0	15.79	15.79	15.79	36.84	5.26	21.05

TABLE 11

The initial score and pH values of the storage butter at the 1926 dairy show

SCORE OF BUTTER	AVERAGE pH	SCORE OF BUTTER	AVERAGE pH
94 and above	5.81	90–91	6.01
93–94	6.23	89–90	5.40
92–93	6.26	Below 89	5.50
91–92	6.17		

The proteolytic group of microörganisms were inhibited the least by the percentage of salt in the brine of the butter, and yeasts were more sensitive than molds. There were but three butters whose salt concentration in brine exceeded 20.1 per cent and because of this small number they were not tabulated.

The hydrogen-ion concentration of the 183 samples of butters at the Detroit Dairy Show of 1926 was determined before storage, and the average results are given in table 11.

The pH values were not considered in reference to the score of the butter after storage of ninety days. There was no consistency in the comparison of pH value and the score. Many of the samples of these butters increased in score during this period, irrespective of the pH value or protein hydrolysis. This condition may occur in many lots of butter made from pasteurized cream. In work done at this station (5), it was found, where cream was pasteurized at 185°F. and held in storage there was a gain in score during storage, and at the end of ninety days the butter gained in storage 0.85 points, but at the end of five months the score fell considerably below the initial score. Cream pasteurized at 145°F. and held for twenty minutes gradually lost in score from the beginning of storage until at the expiration of five months; however, the final score was higher than butter made from cream pasteurized at 185°F. The initial lower score of butter made from cream pasteurized at 185°F. was due to the pronounced oily flavor and frequently, a slightly cooked flavor. This oily and cooked flavor became less marked during storage and in many cases entirely disappeared.

Many samples of the National Dairy Show butters increased in score during the storage of ninety days for various causes. Had these butters been stored for one hundred eighty days there would have been undoubtedly a decrease in score. In our restricted study and application of he pH value for these butters, we find that the butters showing a high number of proteolytic type of organisms showed a greater increase in the pH value during storage than did butters with low numbers of proteolytic type organisms.

THE PROTEOLYTIC CHANGES THAT OCCUR DURING THE STORAGE OF BUTTER AND THE EFFECT OF ITS QUALITY

Inasmuch as we have limited our investigation to the proteolytic changes of butter during storage, we will confine our discussion to the facts involved in bringing about these changes and in consequence of this, the causes of the depreciation of the quality of the butters.

In the first part of this investigation graded cream of fair quality was used, part of which was inoculated with certain specific organisms. The results show a distinct difference in protein hydrolysis, especially when active proteolytic types of organisms were used. All stored butters showed some proteolytic changes during storage. It is well known that pasteurization does not destroy all the organisms nor the enzymes. Those organisms which survived, developed during the ripening of the cream and contributed, in a measure, proteolytic enzymes. The addition of the organisms to the pasteurized lots of cream have intensified the proteolytic action in the butter and have caused a distinct additional depreciation of the quality of the butter. There was a marked increase in protein hydrolysis for all lots of inoculated butter, except *B. butyricus*, which produced only a slight increase in soluble nitrogen not precipitated by phosphotungstic acid, but an increase in peptones. The quality of all butter depreciated during storage; however, in addition to the lardy and flat condition of the control samples, the inoculated ones acquired additional defects such as unclean, flat, tallowy, oily, musty, cheesy, etc. It has been quite generally held that the salting of butter tends to preserve butter. The average increase of soluble nitrogen compounds for the control butters was, for unsalted butters 1.72 per cent as nitrogen not precipitated by phosphotungstic acid and 2.44 per cent peptone, while for the salted butter the corresponding increase was 1.97 and 2.53 per cent. The salted butter gave a slightly larger increase, 0.25 and 0.09 per cent. For the inoculated butter, there was a slight increase of nitrogen not precipitated by phosphotungstic acid of 0.09 per cent and a slight decrease of peptones of the not salted and salted butters. These comparative results show unmistakably that salt had little influence on the protein decomposition.

Otto Rahn and co-workers (1) found protein hydrolysis continued in stored butter having a brine concentration of 21.6 per cent of salt. When stored at −6°C. the salted butter increased 64 per cent in soluble nitrogen not precipitated by phosphotungstic acid, while the unsalted butter increased 36 per cent. At +6°C. there was a decided increase in soluble nitrogen in both salted and unsalted butter.

In the study of the microörganisms it was found that the proteolytic types were more resistant than the acid type, yeasts, and molds and that an increase in the proteolytic type during storage decreased the quality of the butter. The effect of the increase or decrease on the score of butters is significant, the summary of which is in close correspondence with the results of the proteolytic changes in these butters during storage.

The two primary causes which increase or decrease the development of bacteria and other microbial organisms are temperature and reaction of the media or the pH value. For enzymes the same conditions cause an increase in activity or an inactivation or final destruction. This is a natural consequence inasmuch as all enzymes are elaborated by living cells.

There is little doubt but that the protein hydrolysis of the butter during storage is due to the proteases derived from the organisms present in the cream and consequently in the corresponding butter. Fuhrman (9) reports numerous cases of undisputed action of bacterial proteases, peptonizing casein and gelatin. Also in many instances the tryptic nature of the enzyme predominated. Most of this work had been done only in a qualitative manner so the results are therefore indicative rather than conclusive. Waksman (10) quotes numerous cases of the existence of bacterial enzymes and their proteolytic actions, peptic, tryptic, and ereptic and for a more complete hydrolysis the action of peptidase (erepsin) becomes manifest. Each of these groups of enzymes have an optimum pH value, the pepsinase in the acid region, tryptase and peptidase from near the neutral to a slight alkaline region.

The effect of the pH value, which measures the degree of dissociation or the hydrogen ion concentration, is of greater importance in its effect on the keeping qualities of butter than generally supposed. At certain pH values the proteins are less dissociated, show the least hydration, lowest solubility, and thus be least subject to enzymic action. This pH value may be the optimum one for enzyme action.

Euler (11) in summarizing the present state of knowledge of the proteases of bacteria arrived at the following. All investigated bacteria contain proteases, but concerning the number of these enzymes there is no agreement. The often expressed view that in many bacteria no peptidase occurs, may depend upon imperfect methods. In modern investigations it was found that by suitable acidity there is always peptidase action. In an acid solution it may on the contrary come to a standstill early in the region when pepsinase action was effected, for the action of the occurring peptidase is considerably weakened. It is apparent that we have at least two well defined groups of bacterial proteolytic enzymes, pepsinase and tryptase. It must be noted, however, that the animal proteases have a narrower range of activity both as regards temperature and acidity, than the plant proteases which are quite active over a much greater range.

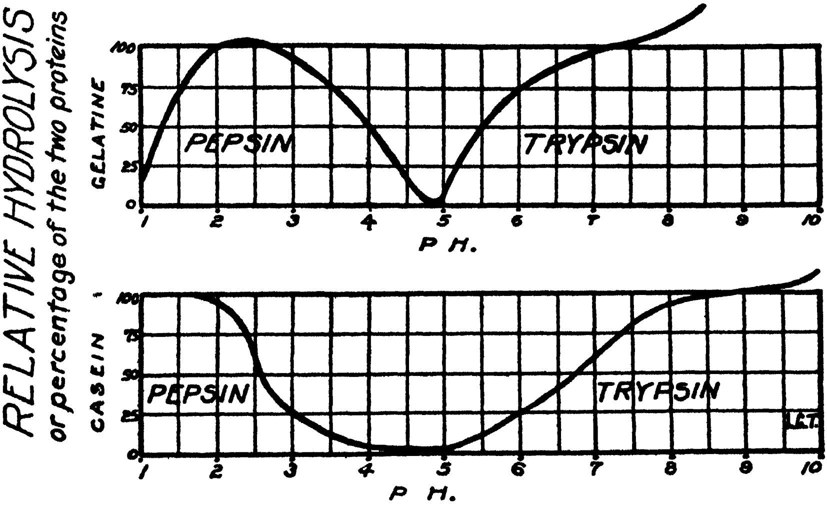


FIG. 1. THE RATE OF HYDROLYSIS OF GELATINE AND CASEIN AT VARYING HYDROGEN-ION CONCENTRATIONS

The pH curves, given in figure 1, are taken from Lehrbuch d. Enzyme (12). They show that the degree of digestion or hydrolysis has a minimum at the isoelectric point of the proteins pH 5. It also shows that there is a striking parallellism of the ionization of the proteins and the velocity of hydrolysis.

From our experimental data (table 4) which showed that butters which entered storage having a pH value of 5 to 6 lost 1 point, while butters which had a pH value of 6 to 7 and 7 plus lost 2.3 and 1.80 points, respectively. From these data and from the discussion of the condition of proteins at the isoelectric point at pH 4.6, this would indicate that butters going into storage should have a pH of 5 to 6 to secure the maximum keeping qualities. The investigations cover only one of the many factors involved, but from our past work and that of others, the proteolytic changes in butter during storage are of primary importance and a control of the proteolytic hydrolysis contributes in a great measure to prevent loss or depreciation of the butters when stored.

REFERENCES

(1) Rahn, Otto, Brown, C. W., and others: Keeping qualities of butter. Michigan Agric. Exp. Sta. (1908–1909).
(2) Fisher, K., and Gruenert, O.: The influence of some preservatives on the keeping qualities of butter. Zeit. F. Untersuchung d. Nahrungs and Genussmittel (1911).
(3) Rogers, L. A., and Gray, C. E.: The influence of acidity of cream in the flavor of butter. Bureau of Animal Industry, Bulletin 114. U. S. Department of Agriculture (1909).
(4) Rogers, L. A., Berg, W. N., Potteiger, C. R., and Davis, B. J.: Factors influencing the change in flavor in storage butter. Bureau of Animal Industry. U. S. Department of Agriculture (1913).
(5) Hunziker, O. F., Spitzer, George, Mills, H. C., and Switzer, H. B.: Purdue University Agric. Exp. Sta., Bul. 208 (1917).
(6) Ferris, J. W.: Some determinations on the soluble nitrogen compounds of cream and butter. Jour. Dairy Sci., vi, 320–329 and 412–427 (1923).
(7) Parfitt, E. H., and Spitzer, George: The quantitative action of enzymes of seven specific organisms upon the proteins of milk and upon gelatin. Jour. Bact., ix, 123–130 (1924).
(8) Ayers, S. H., and Mudge, C. S.: Milk powder agar for the determination of bacteria in milk. Jour. Bact., v, 565–588 (1920).
(9) Fuhrman, Franz: Vorlesungen uber Bakterien-enzyme. Jena (1907).
(10) Waksman, S. A.: Abst. Bact., vi, no. 2 (1922).
(11) Euler, H. v: Chemie der Enzyme, II Teil, 2, Abschuitt, Munchen (1925).
(12) Oppenheimer, Carl: Lehrbuch der Enzyme. Leipzig (1927).

THE USE OF CITRIC ACID AND SODIUM CITRATE IN STARTER CULTURES*

HUGH L. TEMPLETON AND H. H. SOMMER

Dairy Department, University of Wisconsin, Madison, Wisconsin

The flavor of butter made from ripened cream is preferred by many consumers. The starters which are used for this purpose have been the subject of many investigations the main object of which has been to determine the factors that affect the flavor and aroma of the starters. The work of Rogers and Gray (1) and others has clearly shown the harmful effect of a high acidity on the keeping quality of butter, making it important to obtain the flavor and aroma at as low an acidity as possible.

Among the numerous investigations on starters the work of Hammer (2) is of especial value to this paper since he worked with organisms that destroyed the citrates present in milk. In his researches he found two organisms usually associated with *Streptococcus lacticus* in commercial starter cultures. The two organisms which he named *Strep. citrovorous* and *Strep. paracitrovorous* are capable of fermenting citrates and lactates producing volatile acids. Pure cultures of these organisms were found to produce an increased volatile acidity in milk if the citric acid content was increased by the addition of citric acid in varying amounts, however the use of pure cultures was considered impractical under ordinary commercial conditions.

As far as the authors have been able to ascertain the addition of citric acid or citrates to ordinary commercial starters has not been considered.

The researches to be described in this paper were undertaken in order to study the effect of the addition of citric acid and sodium citrate on the volatile acidity produced in commercial starter cultures and also to give a comparison of cultures treated in such

---

* Published with the permission of the director of the Wisconsin Agricultural Experiment Station. Received for publication July 9, 1928.

TABLE 1

The destruction of citrates in the spontaneous souring of milk

SAMPLE NUMBER	CITRATE CONTENT OF MILK							
	Fresh	After						
		24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	168 hours
1	0.197							
	0.200		0.219	0.176			0.109	0.009
	0.195		0.213	0.172			0.103	0.006
	0.178		0 213	0 175				
	0.194							
2	0.191	0.133	0.032	0 012		0.141		
	0.187	0.127	0.130	0 009		0.143		
		0.130	0 050	0 012		0.044		
3	0.203				0 251		0.083	
	0.200	0.118			0 097	0 038	0.080	
	0.200	0.121				0 025	0.078	
	0.197					0.028		
4	0.184	0.109		0 012	0 009		0 006	
	0.184	0.106		0.012	0.006	0 006	0.006	
	0.187	0.106				0.038	0.009	
5	0.175	0 026		0 003	0.009			
	0.178	0.026	0.009	0.003	0.006			
	0.178	0 029	0.006					
6	0.187	0.156		0.006				
	0.187	0.156		0.006				
7	0.191	0.038	0.009	0.006				
	0.187	0.038	0.006	0.006				
	0.187							
8	0.187	0.071						
	0.187	0.071						
	0.184							
9	0.194							
	0.187	(Whole milk)						
	0.191							

Average of all determinations for the fresh milk 0.189.

manner with untreated ones in regard to their suitability for use in buttermaking.

Before taking up these comparisons it was thought advisable to make a brief study of the amount of citric acid present in milk and the rate at which it disappeared as the milk was allowed to sour spontaneously or with pure cultures of organisms. Beau's modification (3) of the method of Denige (4) was used. The milk was, unless otherwise noted, skim milk obtained directly from the separator in the University of Wisconsin creamery. The results obtained by allowing milk to sour spontaneously are given in table 1.

As a check on the method 0.20 per cent of citric acid was added to sour milk in which the citric acid content had been found to be 0.006. Triplicate analyses on the treated milk showed 0.203, 0.210, 0.207 per cent of citric acid which after the deduction of the control leave 0.197, 0.204, and 0.201 respectively.

In table 1 it is interesting to note that in some cases the citric acid content apparently reached a minimum and then increased. After this phenomenon was first noticed, the analyses succeeding the apparent minimum were watched for difference in behavior. It was found that the solution after the addition of permanganate and decolorization with hydrogen peroxide was practically free from precipitate while warm, but upon cooling a fine white precipitate formed. This substance behaved the same as the mercury dicarboxy sulpho-acetone, being soluble in HCl and then reacting with the KCN. This last reaction would indicate that mercury was combined with the unknown material. It was thought best not to try to identify this compound due to lack of time in which to prepare sufficient material of known purity to warrant complete characterization.

In order to study the effect of pure cultures of bacteria upon the citric acid content of milk, cultures of organisms were obtained from the Dairy Bacteriology department. These were used to inoculate sterilized milk made up from milk powder. The flasks were kept at 30°C. and samples were drawn at intervals for analysis, care being taken to prevent contamination when the samples were removed. The high citric acid content of the milk was quite unexpected, but did not seem to affect the rate of disappearance. The results are shown in table 2.

The reduction of the citric acid content by the culture of *Strep. paracitrovorous* was slight so that its contamination was suspected. A fresh culture was obtained from Prof. B. W. Hammer of Iowa and this was compared with the one used before. Results are given in table 3.

TABLE 2

The destruction of citrates by various cultures

SAMPLE NUMBER	CULTURE ORGANISM	CITRATE CONTENT OF MILK				
		Fresh	After			
			48 hours	96 hours	144 hours	192 hours
1	*Strep. citrovorous*	0 251				
		0.242	0.162	0.147	0.147	0.156
		0.245	0.162	0 147	0 147	0.144
		0 245				
		0 248				
		(0 246)*				
2	*Strep. paracitrovorous*		0.130	0.119	0 109	0.105
		0 246	0.127	0 121	0.112	0.106
3	54-V	0 246	0 009	0 000		
			0.007	0.000		
4	59-2	0 246	0 015	0 003		
			0.012	0.003		
5	5-1	0 246	0.048	0 015	0 003	0.003
			0 050	0 012	0.003	0.004
6	103-A	0.246	0.019	0.007		
			0.017	0.009		

* Average.

These results show very clearly that the culture used previously must have contained some other organism. The destruction rate of citric acid is more nearly that given by *Strep. citrovorous* in table 3.

Confirmation of these results was obtained at a later date using pure cultures of the organisms. In table 4, the citric acid content of the milk is given at the intervals indicated, the acidity calculated as lactic acid and per cent of volatile acids when the destruction of citrates had ceased. The method of Hammer (loc. cit.) was used in the determination of the volatile acids. Two hundred and fifty cubic centimeters of milk were treated with 15 to 16 cc. of a normal solution of sulphuric acid and then distilled with steam. One liter of distillate was taken and the acids titrated with N/10 alkali. In the determination of total acid 25 cc. of milk were taken so that the calculation of the percentage of volatile acid was very simple. Six flasks each containing one liter of milk were inoculated, three with each culture, and incubated at 30°C. Fifty cc. of milk was removed for analyses in order to leave as much as possible for distillation. In table 4, therefore each analysis represents one flask. Three portions from each flask were distilled. The acidity of the milk before inoculation was 0.21 per cent calculated as lactic acid.

TABLE 3

The destruction of citrates by various cultures

SAMPLE NUMBER	CULTURE ORGANISM	CITRATE CONTENT OF MILK					
		Fresh	After				
			72 hours	120 hours	168 hours	240 hours	288 hours
1	*Strep. paracitrovorous*	0.216					
		0.219	0.097	0.009	0.003		
		0.219	0.133	0.017	0.003		
		0.217	0.115	0.009	0.004		
2	*Strep. paracitrovorous**	0.213					
		0.216	0.197	0.187	0.165	0.165	0.165
			0.194	0.194	0.172	0.156	0.165
			0.160	0.181	0.147	0.130	

* Note: Culture No. 2, table 2.

The figures in table 4 indicate that *Strep. paracitrovorous* produces a relatively greater amount of volatile acids due to the smaller total acidity whereas if one were to compare the titration figures in cubic centimeters of alkali used this difference is not apparent. The total high acidity of the first culture of *Strep. citrovorous* would give a relatively lower volatile acidity, although the titration figures were somewhat higher than the others.

TABLE 4

The destruction of citrates by Strep. paracitrovorous and Strep. citrovorous and the production of volatile acids

ORGANISM	CITRIC ACID CONTENT				ACIDITY	
	Start	After			Total	Volatile expressed as percentage of total
		72 hours	120 hours	168 hours		
Strep. paracitrovorous	0 207					36.64
	0 203	0 015	0 006		0.44	33.13
	0.207					32.57
	0.203					
	0.208					37 96
	0.203	0 017	0 009		0 42	35.60
	0.203					34 31
	(0.205)*					
						38.84
		0.013	0.015		0 44	32.43
						32.43
						24.54
		0.185	0.159	0.009	0.75	22 20
						24.89
Strep. citrovorous	(0.205)*					
		0.172	0.156	0.012	0.53	30.97
						33.62
						31.67
						30.22
		0 175	0.161	0.026	0.52	28.01
						27.97

* Average.

The work on the citrate content and volatile acidity of milk soured spontaneously and by pure cultures of organisms was a study preliminary to the main object of this work which was to study the effect of adding citric acid and sodium citrate to the commercial starter cultures as put out by the different companies engaged in such work. The work with the commercial starter cultures consisted of a comparison of the total and volatile acidities produced in untreated cultures and cultures treated with either citric acid or sodium citrate and later an organoleptic comparison of such cultures to determine their suitability for practical use.

TABLE 5

Total and volatile acid produced in sterilized milk by commercial starter cultures

FLASK NUMBER	AGE OF CULTURE							
	48 hours		48 hours		48 hours		48 hours	
	Total acidity	Volatile acidity	Total acidity	Volatile acidity	Total acidity	Volatie acidity	Total acidity	Volatile acidity
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.82	24.17	0.88	21.93	0.94	14.65	1.01	12.89
2	0.75	27.02	0.84	21.38	0.94	15.02	0.99	13.69
3	0.90	17.15	0.96	15.49	0.91	15.57	1.01	12.79
4	0.89	17.86	0.95	15.81	0.92	15.51	1.00	13.43
5	0.83	17.66	0.92	15.58	0.95	13.58*	0.98	13.59
6	0.83	18.05	0.95	16.82	0.96	15.15	1.00	14.24
7	0.90	17.93	0.99	13.23	1.02	13.15	1.01	11.93
8	0.89	18.26	0.99	12.51	0.98	13.61	1.00	11.91
9	0.84	18.98	0.92	17.25	1.00	16.06	0.99	13.33
10	0.84	18.76	0.89	16.72	0.97	16.50	1.01	13.45
11	0.82	17.70	0.91	18.97	0.99	16.24	0.98	13.92
12	0.84	17.10	0.88	19.14	0.92	14.45*	0.92	14.73
13	0.85	18.08	0.88	17.94	0.93	14.32	1.00	15.66
14	0.84	17.09	0.88	17.47	0.93	14.25	1.00	15.18
15	0.84	18.71	0.88	19.30	0.93	15.12	0.99	14.85
16	0.83	18.32	0.88	19.42	0.94	15.36	0.97	15.66

* Steam connection broke after the acid had been aded to the milk which may account for the rather wide variation between 5 and 6, and 11 and 12.

Eight different cultures were used supplied through the courtesy of four laboratories. These were prepared for use in the usual manner. In order to be sure that there would be a source for renewal in case any should become contaminated duplicates were carried in sterile milk. Cultures for analyses were kept in sterile milk for a time (tables 5 and 6) but later milk heated to approximately 190°F. for an hour was substituted. Into 500 cc. sterile Erlenmeyer flasks 300 cc. of milk were placed after heating to the temperatures indicated and thoroughly cooled. The cultures in sterile milk were kept in 125 cc. flasks each containing about 60 cc. of milk. The transfers were made at intervals of one to two days depending upon the speed with which the analyses were completed, using about 2 per cent of inoculum.

Analyses of these cultures were made as previously described for both total and volatile acidity. After the removal of the portion for the inoculation of the freshly prepared milk the old cultures were kept cold until they could be distilled. Conditions were such that it was very difficult to carry on more than two distillations at one time. This necessitated the keeping of some of the cultures overnight unless one could get the fresh milk ready for transferring cultures before noon. When this became possible due to the earlier arrival of the milk at the creamery analyses were

TABLE 6

Total and volatile acid produced in sterilized milk with and without added citric acid by commercial starter cultures

(0.2 per cent citric acid added to even numbered flasks)

FLASK NUMBER	AGE OF CULTURE							
	48 hours		48 hours		48 hours		48 hours	
	Total acidity	Volatile acidity	Total acidity	Volatile acidity	Total acidity	Volatile acidity	Total acidity	Volatile acidity
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	1.01	13.39	0.99	13.35	0.94	13.19	1.02	13.71
2	1.08	22.84	1.05	21.37	0.99	23.15	1.08	23.29
3	0.97	13.44	0.98	12.36	0.91	13.15	0.99	14.13
4	1.03	21.19	1.04	21.22	1.01	20.82	1.01	22.43
5	1.00	13.13	0.95	13.08	0.94	13.75	0 99	15.91
6	1.06	22.48	1.07	21.55	1.01	21.71	1.05	24.50
7	1.02	13.55	0.98	14.43	0.97	13.16	0.98	15.13
8	1.07	21.04	1.05	22.78	1.04	20.03	1.06	26.68
9	0.98	15.77	0.92	14.65	0.89	16.03	0.94	15.10
10	1.05	23.87	1.06	22.07	1.04	21.74	1.10	21.80
11	0.98	14.43	0.96	12.82	0.95	13.71	1.02	14.81
12	1.03	22.91	1.05	22.16	1.02	22.23	1.09	24.61
13	0.97	13.37	0.97	12.70	0.93	15.31	1.01	14.09
14	1.02	21.55	1.07	22.65	1.00	22.35	1.06	21.73
15	0.99	13.13	0.95	14.91	0.90	13.25	1.01	13.03
16	1.04	21.63	1.07	24.61	1.04	20.83	1.04	23.70

made daily. All incubation was done in an electrically heated and controlled incubator adjusted for 70°F. ± 0.2°F.

The first series of analyses, table 5, were made on sterilized milk to which nothing had been added. The second series, table 6, was sterilized milk to which 5 cc. of a solution of citric acid were added to one of each pair of duplicate flasks. The strength of the solution was such that the citric acid content of the milk was increased two-tenths of 1 per cent. The third series, table 7, was similar to the second except the milk was pasteurized at 190°F. for one hour. In the fourth series, table 8, sodium citrate was substituted for the citric acid in a solution of equivalent citric acid content. In order to determine if there was any marked change in the volatile acid production with repeated transfers one culture was kept growing in milk to which citric acid was

TABLE 7

Total and volatile acid produced in pasteurized milk with and without added citric acid by commercial starter cultures

(0.2 per cent citric acid added to the milk in even numbered flasks)

FLASK NUMBER	AGE OF CULTURE					
	48 hours		24 hours		48 hours	
	Total acidity	Volatile acidity	Total acidity	Volatile acidity	Total acidity	Volatile acidity
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.97	14.78	0.97	16.67	1.02	13.23
2	1.07	22.36	0.97	25.11	1.10	19.31
3	0.93	13 66	0.97	13.37	0.95	13.98
4	1.03	20.28	1.03	20.49	1.06	19.73
5	0.97	14.72	0 97	12.60	0 99	14 20
6	1.02	23.05	0 97	20 89	1.05	20.79
7	1.00	14 35	0 97	15 33	0.97	14 16
8	1.06	21.93	0 93	28.06	1 05	23.02
9	0.89	19.02	0 88	15.53	0 96	14.06
10	0.96	27 33	0 90	26.28	1.07	23.42
11	1.05	14.78	1.02	15.65	1.00	12.69
12	1 07	24 38	1 04	23.75	1.08	20 90
13	0.95	14.20	0.99	14.04	1.01	13.46
14	1.04	22.28	0.98	21.50	1.06	22.37
15	0.95	13.13	0.98	13.42	0.99	15.83
16	0 99	23.42	0 98	22 09	1.11	22.96

TABLE 8

Total and volatile acid produced in pasteurized milk with and without added sodium citrate by commercial starter cultures

(0.2 per cent citric acid added as sodium citrate to the milk in even numbered flasks)

FLASK NUMBER	AGE OF CULTURE							
	48 hours		48 hours		24 hours		48 hours	
	Total acidity	Volatile acidity	Total acidity	Volatile acidity	Total acidity	Volatile acidity	Total acidity	Volatile acidity
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.93	17.66	0.91	13.99	0.91	17.48	0.97	17.44
2	1.06	22.51	1.06	20.92	1.00	25.02	1.05	25.08
3	0.97	14.76	0.95	13.50	0.90	18.92	0.94	14.73
4	1.02	21.37	1.06	20.00	0.99	24.46	1.05	22.34
5	0.97	12.93	0.95	14.72	0.88	17.36	0.93	14.71
6	1.05	19.52	1.05	21.33	0.97	23.97	1.02	21.77
7	0.73	20.94	0.87	15.85	0.56	21.16	0.88	15.82
8	1.03	22.94	1.00	21.51	1.00	23.31	1.03	22.59
9	0.93	14.40	0.92	17.33	0.86	19.41	0.97	16.89
10	1.02	22.16	1.00	23.69	0.85	25.72	1.00	25.79
11	0.99	16.52	1.00		0.95	14.90	1.01	13.93
12	1.07	22.83	1.09		0.99	22.57	1.18	20.58
13	0.98	14.36	0.99	14.67	0.94	15.88	1.00	14.00
14	1.08	21.27	1.11	21.46	0.98	23.63	1.10	20.66
15	0.98	13.09	0.98	15.66	0.94	14.47	0.97	14.87
16	1.06	19.80	1.08	22.43	1.00	22.27	1.05	22.09
10C*					0.94	26.35		
	24 hours		24 hours		24 hours		48 hours	
1	0.84	19.60	0.83	14.70	0.93	16.26	1.01	15.44
2	0.95	24.66	0.97	20.67	1.04	19.44	1.03	21.11
3	0.90	15.62	0.89	15.37	0.90	14.58	0.97	13.48
4	0.97	20.97	0.97	20.63	1.00	20.68	1.07	18.02
5	0.89	14.76	0.86	13.54	0.93	15.37	0.99	15.22
6	0.99	20.51	0.95	20.07	1.00	23.07	1.07	21.22
7	0.73	18.12	0.73	16.91	0.88	16.23	0.97	17.69
8	0.75	23.68	0.82	25.28	0.80	22.52	0.94	
9	0.84	18.15	0.90	15.06	0.94	14.60	0.95	15.51
10	0.91	24.54	0.81	23.96	0.97	22.15	0.94	
11	0.94	15.69	0.88	14.94	0.94	17.75	1.00	14.46
12	0.97	25.77	0.95	21.89	1.02	24.19	0.95	
13	0.91	14.72	0.88	14.71	0.92	15.98	0.99	14.31
14	0.95	22.27	0.93	21.82	1.00	20.83	1.10	20.13
15	0.94	14.46	0.89	16.18	0.95	14.05	1.01	14.21
16	0.98	21.81	0.95	25.45	1.02	19.50	1.07	20.57
10C	0.88	24.67	0.90	24.98			0.92†	25.07

* 10C a culture from flask no. 10 that was kept in milk to which citric acid was added (0.2 per cent) after other cultures were changed to milk having sodium citrate added.

† Twenty-four hour culture.

added after the others had been changed to sodium citrate. The results are given in table 8.

Averaging all the results given in the previous tables, we find that 183 analyses of cultures grown in milk without any additions averaged 15.43 per cent volatile acids, 32 cultures grown in sterilized milk with 0.2 per cent citric acid added gave 22.42 per cent, 28 cultures grown in pasteurized milk with 0.2 per cent citric acid added averaged 23.10 per cent volatile acids and 60 cultures grown in pasteurized milk with 0.2 per cent citric acid added as sodium citrate averaged 22.21 per cent volatile acids. From these figures it is evident that the addition of 0.2 per cent citric acid either as the acid or as its sodium salt increases the amount of volatile acids produced approximately 50 per cent.

Examination of the cultures before making transfers showed that all of them produced very satisfactory curds before the addition of either citric acid or sodium citrate. The addition of the former tended to give a rather weak curd which would whey off if the culture was allowed to incubate for more than twenty-four hours. The sodium citrate treated milk gave just as good a curd as the original cultures before the addition of the salt. It was also noted that the citric acid cultures grown in sterilized milk showed a greater tendency to whey off than when they were grown in pasteurized milk. When such cultures were removed from the incubator as soon as possible after curdling was complete and thoroughly chilled, there was little if any difference between them and untreated milk that was handled similarly.

Since starters are selected mainly by their flavor, it was thought advisable to have starter cultures representing the above treatments place by disinterested judges. This was done on two different occasions, four days apart. The first time there were six men from the dairy .department staff and the second time five men, two from the resident staff, the extension worker of the department, the secretary of the Wisconsin Buttermakers' Association and one of the inspectors from the Dairy and Food Commission. Scoring of the cultures was not requested in any case, only the indication of preference of the first three from among the four or five cultures that were prepared from each of the originals.

TABLE 9

Selection of starter cultures treated with citric acid or sodium citrate and also untreated

CULTURE	JUDGE'S CHOICE	JUDGE'S NUMBER					
		1	2	3	4	5	6
A	1	FSC	FSC	FSC	OSC	C	OSC
	2	FCA	OSC	CA	FCA	FCA	FSC
	3	CA	FCA	OSC	C	CA	FCA
B	1	OSC	OSC	CA	CA	OSC	C
	2	CA	FCA	OSC	OSC	CA	CA
	3	FCA	C	FCA	FCA	C	OSC
C	1	FSC	FSC	OSC	FCA	C	OSC
	2	OSC	FCA	FSC	C	FSC	C
	3	FCA	OSC	FCA	FSC	FCA	FCA
D	1	OSC	OSC	CA	CA	CA	OSC
	2	CA	CA	OSC	OSC	OSC	FCA
	3	FCA	C	FCA	C	C	CA
E*	1	FCA	FCA	OSC	FCA	C	OCA
	2	OSC	OSC	FCA	OSC	OCA	FCA
	3	OCA	OCA	OCA	C	FCA	OSC
F	1	OSC	FSC	OSC	FSC	FCA	OSC
	2	FSC	OSC	FSC	OSC	C	FSC
	3	FCA	C	FCA	C	OSC	FCA
G	1	FCA	FCA	OSC	C	FCA	CA
	2	OSC	C	FCA	FCA	OSC	FCA
	3	CA	OSC	CA	OSC	CA	OSC
H	1	OSC	FSC	FSC	FCA	FCA	OSC
	2	FSC	C	OSC	FSC	C	FSC
	3	FCA	OSC	C	C	OSC	FCA

* Series E was the only one containing an old citric acid culture.

Summary of above table

TREATMENT	FIRST CHOICE	SECOND CHOICE	THIRD CHOICE	NUMBER OF CULTURES
Control	5	6	11	8
Fresh citric acid culture	10	11	17	8
Citric acid (two transfers)	6	6	6	4
Old citric acid	1	1	3	1
Fresh sodium citrate culture	9	9	1	4
Old sodium citrate culture	17	15	10	8

In the groups presented on the first day there were six different treatments represented, the letters in parentheses being the abbreviations used in table 9, as follows:

Control, (C) untreated milk.
Milk to which 0.2 per cent citric acid had been added only at the time the culture was started (FCA).
Milk that had two transfers with 0.2 per cent citric acid added each time (CA).
Milk that had 0.2 per cent citric acid added for a number of transfers (OCA).
Milk having 0.2 per cent citric acid as sodium citrate added the preceding day (FSC).
Milk that had 0.2 per cent citric acid added as sodium citrate for a number of transfers (OSC).

All the cultures were incubated at 70°F. for about twenty-four hours and were cooled before the selections were made. None of the cultures showed any appreciable amount of whey, all were free from gas and all were thoroughly shaken before the men were allowed to make their selections. The results of this are shown in table 9.

Before the second judging, the culture that had been grown in citric acid was lost. To take the place of this culture in series E a fresh culture was prepared to which 0.4 per cent of citric acid was added. The cultures that had been grown through two transfers with citric acid added for the previous time were kept in series A, B, D and G and were transferred at the same time as the others. The same treatments with sodium citrate were used as for the previous time and also fresh cultures containing 0.2 per cent citric acid. The same symbols are used to show the additions except that in series E the percentage of added citric acid is indicated.

In table 10 judge number one was also number one in the other, while judge number two was number three before. The remaining three were not present at the time the first selections were made.

Skim milk taken directly from the separator was pasteurized,

TABLE 10

Selection of starter cultures

CULTURE	JUDGE'S CHOICE	JUDGE'S NUMBER				
		1	2	3	4	5
A	1	FSC	FSC	FCA	FSC†	C
	2	FCA	C	FSC	FCA	OSC
	3	CA	OSC	C	OSC	FSC
B	1	OSC	FSC	OSC	OSC	OSC*
	2	FSC	CA	C	C	C
	2	CA	C	FSC	CA	FSC
C	1	FSC	OSC*	OSC†	FSC	C
	2	OSC	FSC	FSC	OSC	FCA
	3	FCA	C	FCA	FCA	OSC
D	1	OSC	OSC	CA	CA	FCA
	2	CA	CA	OSC	OSC	OSC
	3	FCA		C	C	CA
E	1	FCA 0.4%	FCA 0.4%	FCA 0.4%	FCA 0.4%	FCA 0.4%
	2	FCA 0.2%	FCA 0.2%	C	FCA 0.2%	C
	3	C	C	FCA 0.2%	C	FCA 0.2%
F	1	FSC*	FSC	C	FSC	C
	2	OSC	OSC	FSC	OSC	FSC
	3	FCA	FCA	OSC	FCA	OSC
G	1	CA	FCA	OSC*	FCA*	C†
	2	OSC	OSC	FCA	C	OSC
	3	FCA	CA	C	CA	FCA
H	1	OSC†	FSC†	FSC	FCA	FCA
	2	FSC	C	C	C	FSC
	3	FCA	FCA	FCA	OSC	C

* Indicates first choice in the entire group.

† Indicates second choice in the entire group.

Summary of these results

TREATMENT	FIRST CHOICE	SECOND CHOICE	THIRD CHOICE	NUMBER OF CULTURES
Control	5	10	10	8
Fresh citric acid culture 0.2 per cent	6	7	14	7
Fresh citric acid culture 0.4 per cent	5			1
Citric acid	3	3	6	4
Fresh sodium citrate culture	11	8	3	5
Old sodium citrate culture	10	12	6	8

cooled, inoculated with the different cultures and immediately after the addition of the chemicals to be studied incubated at 70° F. for eighteen hours. At the end of this time, the cultures were removed and well cooled. Examination showed practically no whey on any of the cultures and in only one instance was there any indication of a broken curd. All the curds were thoroughly broken up before they were submitted for inspection.

At this time the judges were asked to indicate their first and second choice from among the entire group, as well as to place three in each series in the order of their preference. The results are given in table 10.

It is interesting to note that in no case did any culture receive preference over the others by a majority of the men doing the placing. In series C the old sodium citrate culture was the first choice among the entire group for judge number two and second choice for judge number three. While three of the preferences were in series G, the cultures were all different.

These results would indicate that the use of citric acid or sodium citrate with milk intended for starters produces very satisfactory results and in most cases the treated cultures were preferred to the untreated ones.

The use of citrated starters in buttermaking will be discussed in another paper in which the initial score and the keeping quality of the butter made with treated and untreated starters will be presented.

SUMMARY

1. Under ordinary conditions the citric acid content of milk disappears in two to four days.

2. If milk is allowed to remain after the disappearance of the citric acid and then analyzed by the method used, another substance appears which behaves similarly to the compound formed by the mercury and citric acid. The nature of this material is unknown.

3. The addition of either citric acid or an equivalent amount of sodium citrate to milk increases the volatile acidity produced by approximately 50 per cent and does not increase the total acidity more than 10 per cent.

4. In most cases and with different men, cultures to which citric acid or sodium citrate was added were preferred to the untreated ones from the same original culture.

5. From this work and that to be presented later it would seem that as satisfactory results are obtained when citric acid or its equivalent of sodium citrate was added to the milk only at the time of inoculation as when the culture is carried in milk having these reagents added at each transfer.

In conclusion the authors wish to express their thanks to the Charles Pfizer and Company, Inc. for giving the fellowship under which this work was done. The commercial cultures used were supplied by the Ohio Dairy and Food Laboratory, the Elov Ericsson Laboratory, the Dairy Laboratories of Philadelphia, and the Chr. Hansen Laboratories. The cultures were placed in order of their preference by Messrs. L. C. Thomsen, H. T. Sondergaard, H. C. Larson, A. T. Bruhn, C. W. Vilbrandt, L. Germain, L. L. Bishop, and W. B. Sarles.

REFERENCES

(1) ROGERS, L. A., AND GRAY, C. E.: The influence of the acidity of cream on the flavor of butter. Bur. of Animal Ind. Bul. 114 (1909).

(2) HAMMER, B. W., AND CO-WORKERS: Iowa State College, Res. Bul. 55, 63, 65, 66, 67, 80, 81, 85.

(3) BEAU: Dosage de l'acide citrique dans le lait. Revue Generale du Lait, iii, 385 (1903).

(4) DENIGE: Sur de nouvelles classes de combinaisons mercurio-organiques et sur leurs applications. Annales de Chimie et de Physique, xviii, 382 (1899).

A STUDY OF THE FACTORS AFFECTING THE GROWTH OF DAIRY HEIFERS

PART II*

C. B. BENDER AND J. W. BARTLETT

Department of Dairy Husbandry, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey

INTRODUCTION

In 1924 (1), this station reported on the factor of nutrition, with special attention to its effect on body weight and skeletal growth on 28 calves of different breeds which were divided into three groups according to the age at which they were weaned.

The first group was weaned between thirty and forty days of age. The second group was handled similarly to group I, but the calves were weaned at forty to fifty days of age. The third group was weaned at thirty to forty days of age, as were the animals in group I, but black strap molasses was added to the ration during the greater part of the feeding trial.

This experiment demonstrated that calves could be successfully raised on a dry grain mixture when weaned from a milk diet at from thirty to fifty days of age. However, from a study of the graphs, it could readily be seen that the calves of all breeds lost rapidly in weight normality and less rapidly in height normality until they were from sixty to one hundred and eighty days of age. After this period of rapid loss slow recovery was made in weight and height normality until at seven hundred and twenty days of age, 50 per cent of the heifers were normal or above normal in weight and 42 per cent were normal or above normal in height.

PURPOSE OF EXPERIMENT

Feeling that the ration as used in the preceding experiment although fundamentally sound was lacking in palatability and possibly in amounts of some of the amino acids essential for

---

* Received for publication June 23, 1928.

growth, this problem was therefore further investigated to determine, (*a*) the best possible ration to feed dry, which would produce 100 per cent normality in calves at one hundred and eighty days of age, (*b*) to find the earliest age at which calves could be weaned from a milk diet, (*c*) to reduce the cost of raising calves to six months of age.

METHOD OF PROCEDURE

Animals used

At first all purebred calves dropped in the Experiment Station herd were used. This proved very unsatisfactory because of the extreme variation in the age of the animals and because too many other factors were introduced which effected the successful interpretation of results. To overcome this, grade Holstein calves were secured from the Walker-Gordon Laboratories at Plainsboro, New Jersey. By so doing, groups could be used in which most of the animals were born on the same day or within one or two days of each other. The only drawbacks in using these animals were first the comparison of Holstein grade measurements to the Eckle standards for purebreds and secondly 70 per cent of the animals had scours when secured for the experiment at the age of one week.

Rations used and feeding method

The calves used from the Experiment Station herd were left with their dams for thirty-six hours and then placed in individual pens where milk was fed three times a day in amounts varying according to the individual calf. Whole milk was fed alone for the first week. As the Walker-Gordon calves were usually one week of age when delivered, all calves were treated the same from one week until they were accustomed to the grain mixture and alfalfa hay, it being in front of them at all times. At twenty-one days of age a reduction was started in the whole milk feeding and the calves were allowed free access to water. At thirty days of age the whole milk was discontinued and the calves were subsisting on the dry grain mixture, alfalfa hay and water.

The grain was weighed into pails in the morning, placed before the individual calves and with the earlier group weighed amounts of alfalfa hay was placed in racks in the individual pens. The calves were allowed free access to water at all times. The following morning if any grain or hay remained, it was weighed back so that accurate feed records were kept. The calves were turned out in a lot for exercise unless the weather was very bad. The animals received the experimental feed until they were six months of age because the first six months of a calf's life seems to be the most critical. Wood shavings were used for bedding because there was less tendency among the animals to eat it.

Measurements

Weights and measurements were taken on the ninth, tenth and eleventh days of each period, the average of these three was considered as of the tenth day. In measurement, the height at withers was the only one used in this experiment.

Photographic measurements were also taken on the fifth day of each month so that a photographic history of each animal was recorded. These photographs were taken from a fixed point against a fixed background. The animals were in the same relative position for each picture and at the same distance from the camera.

All weights and height measurements were compared with the normal growth figures developed by Eckles (2), and are expressed in percentage of the normal for weight and height.

EXPERIMENTAL WORK

Group I

As milk was the normal diet for all animals until the dams forcibly weaned their offspring, it was thought that milk in some form should be included in the dry grain mixture. For this reason the following ration was used; 20 pounds of yellow corn meal, 10 pounds of wheat bran, 20 pounds of skimmilk powder, 30 pounds of ground oats, 20 pounds of linseed oil meal, 1 pound finely pulverized steam bone meal, 1 pound of finely pulverized limestone and 1 pound of salt. This combination of minerals will hereafter be designated as 3 per cent minerals.

Thirteen animals were used in this group, all purebreds, 8 being Holstein, 3 Ayrshires and 2 Jerseys. The curves were plotted by breed and then the final curve was plotted averaging the thirteen animals so that a more accurate comparison could be made with the grades which were used in the other groups. The animals on this ration had appeared thrifty, strong and consumed the grain mixture with apparent relish although they were subject to a cough which might have been due to the ration being too finely divided.

The average height percentage at six months of age was 97.7 and their weight percentage at the same age was 92.4. At three hundred sixty days of age they were 102.1 per cent normal for height and 114.2 per cent normal for weight. This showed that the animals fed this ration were not stunted in any way. The main drawback to this ration was the high price of skimmilk powder.

Group II

This group consisted of a purebred Holstein, one purebred Ayrshire and three grade Shorthorns. These animals were fed a ration consisting of 25 pounds of yellow corn meal, 37.5 pounds of ground oats, 12.5 pounds of wheat bran, 25 pounds of linseed oil meal, 3 per cent minerals and 2 cc. of 0.2 per cent hydrochloric acid per pound of grain consumed. The reason for using the hydrochloric acid was that it possibly might aid in the breaking down of the proteins during digestion.

These animals averaged 96.2 per cent normal for height and 93.5 per cent normal for weight at one hundred eighty days.

Group III

This group consisted of two grade Holsteins and was fed a straight cereal mixture of 25 pounds yellow corn meal, 37.5 pounds ground oats, 12.5 pounds wheat bran, 25 pounds linseed oil meal and 3 per cent minerals. This group consumed more grain while on the experiment than any other group of animals and at one hundred eighty days of age averaged 97.3 per cent normal in height and 97.4 per cent normal in weight.

Group IV

There were 19 grade Holsteins fed this ration but under different conditions so they will be designated as follows: IV A, IV B, IV C, IV D. They were all fed a grain mixture consisting of 25 pounds yellow corn meal, 37.5 pounds ground oats, 12.5 pounds wheat bran, 12.5 pounds linseed oil meal, 12.5 pounds soluble blood flour and 3 per cent minerals.

Group IV A received all the grain they could consume and good alfalfa hay. This group of seven animals averaged 100 per cent normal in height and 101.2 per cent normal in weight at one hundred eighty days of age.

Group IV B consisting of four animals received the grain twice a day but in limited amounts and the alfalfa hay fed was of the poorest possible quality. This group averaged 91.8 per cent normal in height and 72.5 per cent normal in weight.

Group IV C consisting of four animals received the grain once a day in limited amounts with the extremely poor hay and they averaged 95.1 per cent normal in height and 80.8 per cent normal in weight.

Group IV D consisting of four animals received the grain in more liberal amounts than the two preceding groups, with the very poor hay, and they averaged 99.8 per cent normal in height and 88.6 per cent normal in weight. This grain mixture was again checked with six grade Holsteins using good, green Western alfalfa, and while the animals have only been on the straight grain mixture for four months, their curves are much better than those in group IV A. The animals at five months of age average 103.3 per cent normal for height and 109 per cent normal for weight.

Group V

This group consisting of four grade Holsteins were on feeding trial at the same time as group IV A. They were fed a mixture of 20 pounds of yellow corn meal, 10 pounds of wheat bran, 35

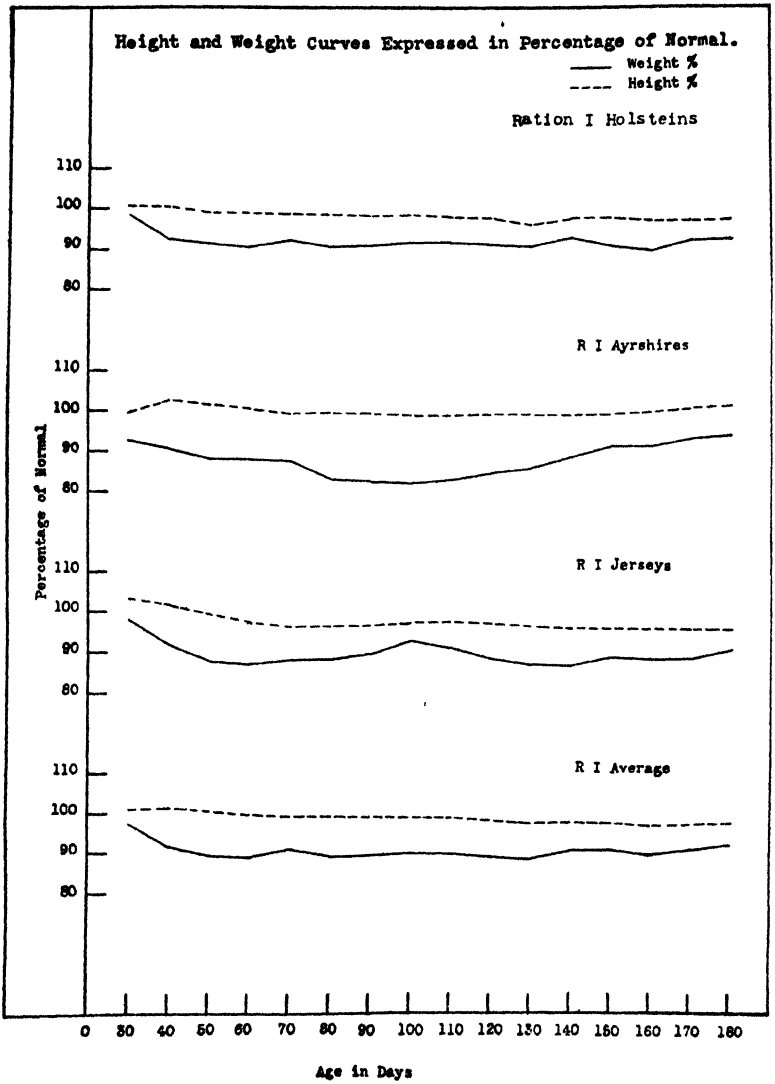

Height and Weight Curves Expressed in Percentage of Normal.
Weight %
Height %
Ration I Holsteins
R I Ayrshires
R I Jerseys
R I Average
Percentage of Normal
110
100
90
80
0
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
Age in Days

FIG. 1

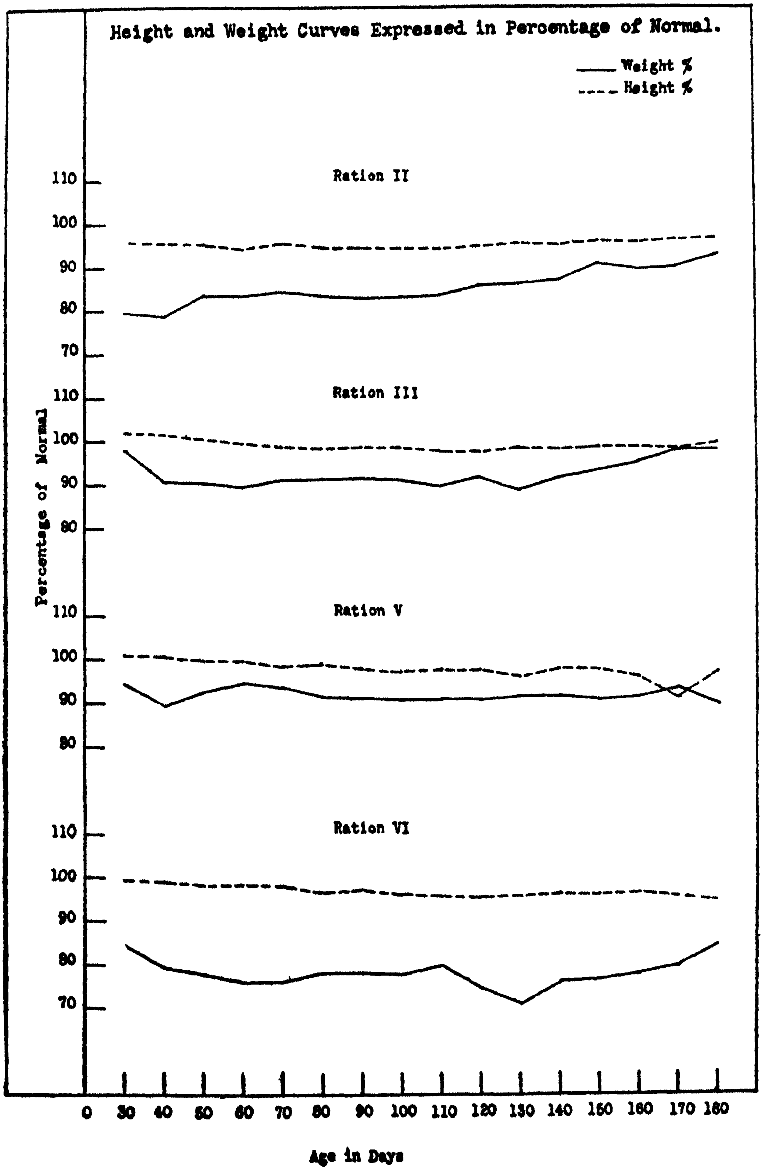

Height and Weight Curves Expressed in Percentage of Normal.
Weight %
Height %
Ration II
Ration III
Ration V
Ration VI
Percentage of Normal
110
100
90
80
70
Age in Days
0
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180

FIG. 2

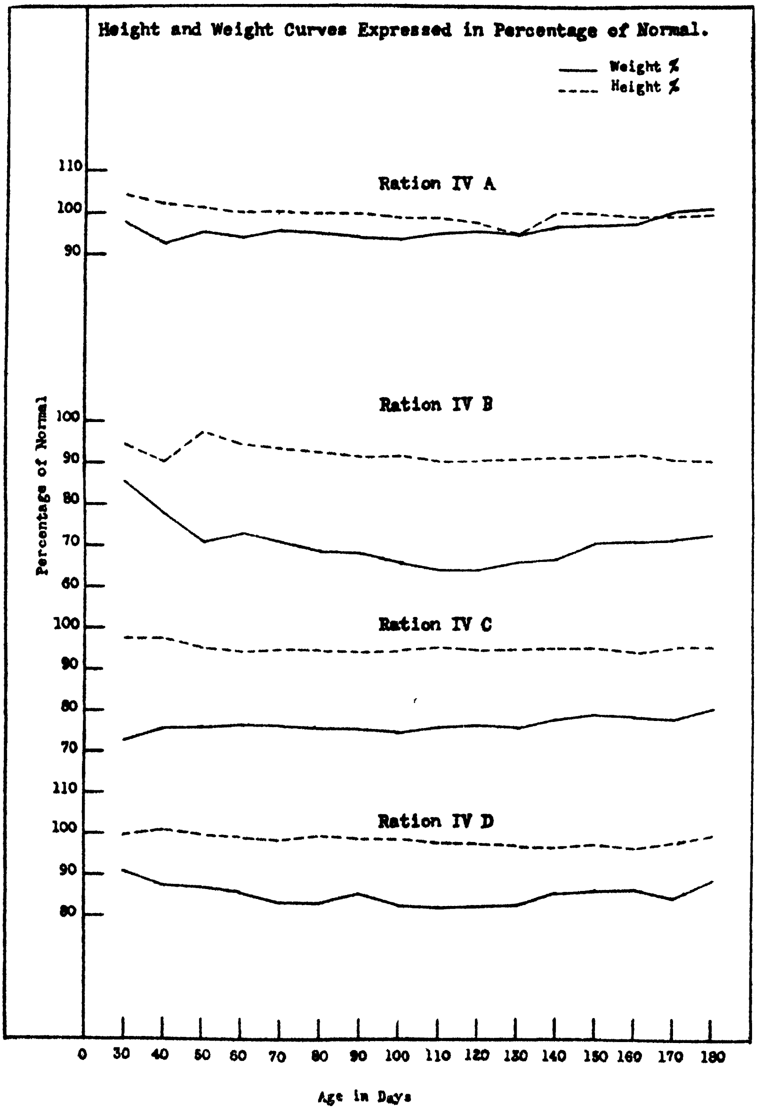

Height and Weight Curves Expressed in Percentage of Normal.
—— Weight %
---- Height %
Ration IV A
Ration IV B
Ration IV C
Ration IV D
Percentage of Normal
110
100
90
100
90
80
70
60
100
90
80
70
110
100
90
80
0
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
Age in Days

FIG. 3

pounds of ground oats, 15 pounds of skimmilk powder, 10 pounds of soluble blood flour, 10 pounds of linseed oil meal and 3 per cent minerals. They averaged 96.7 per cent normal in height and 89.6 per cent normal in weight at one hundred eighty days of age.

Group VI

This group originally consisted of four grade Holsteins. Two of them were so weak that one died while on whole milk and the other died shortly after it was on the dry grain mixture. These animals were on feeding trial at the same time as groups V and IV A. The ration was same as that fed to group IV with the addition of 2 cc. of 0.2 per cent HCl per pound of grain. The two surviving animals averaged 94.7 per cent normal in height and 88.5 per cent normal in weight.

TABLE 1

Tabulation of rations for direct comparison

	I	II	III	IV	V	V
Mineral	3	3	3	3	3	3
Corn	20	25	25	25	20	25
Bran	10	12 5	12 5	12.5	10	12.5
Oil meal	20	25	25	12.5	10	12 5
Oats	30	37 5	37.5	37.5	35	37.5
S. M. powder	20	0	0	0	15	0
Blood flour	0	0	0	12 5	10	12 5
HCl	0	+	0	0	0	+

DISCUSSION OF RESULTS

Practically all of the calves on the dry grain mixture slowed up in weight growth the first ten days and sometimes this lag period lasted for the first thirty days on the rations. This was noticeable especially in rations I, III and VI. Rations II and IV showed the best weight curves. It would seem natural that there would be a slowing up in the rate of growth in weight due to the drastic change in feeding methods for at least the first month on dry grain mixtures.

The calves on ration I showed a tendency to scour at times

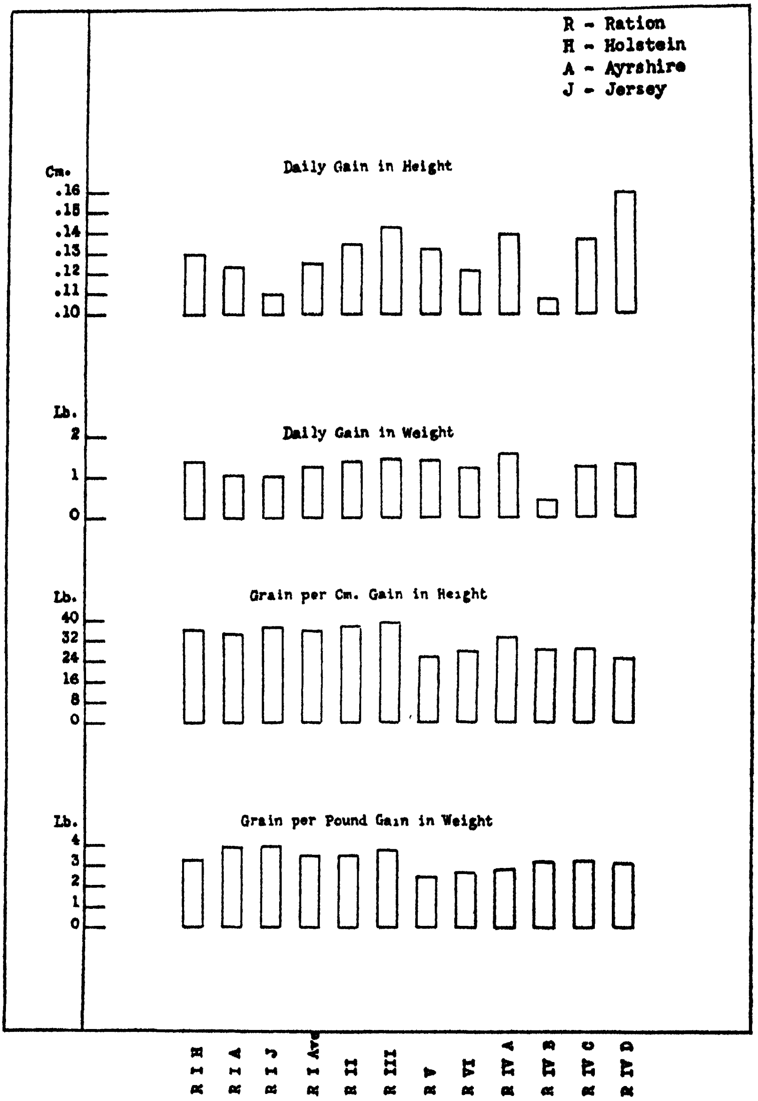

R - Ration
H - Holstein
A - Ayrshire
J - Jersey
Daily Gain in Height
Cm.
.16
.15
.14
.13
.12
.11
.10
Daily Gain in Weight
Lb.
2
1
0
Grain per Cm. Gain in Height
Lb.
40
32
24
16
8
0
Grain per Pound Gain in Weight
Lb.
4
3
2
1
0
R I H
R I A
R I J
R I Av
R II
R III
R V
R VI
R IV A
R IV B
R IV C
R IV D

Fig. 4

although it did not effect their general appearance. This lot of calves however, appeared to carry more flesh than any group of calves but from weight comparisons with other groups it appeared to be soft flesh.

Group IV A was the best from almost every standpoint. In the first place the coats of the animals had a bloom and they showed the dairy temperament of older dairy animals. They did not have the beefy appearance of milk fed calves. They were well middled and consumed their ration with relish. These calves had a very hard flesh, and many good judges of calf weights underestimated the weights of these animals from fifty to one hundred pounds while they were under six months of age. This group made an average daily gain in weight of 1.56 pounds for the one hundred fifty days on the dry grain mixture with a feed consumption of 2.92 pounds of grain per pound gain in weight. This group was the only one which averaged 100 per cent normal in both height and weight at one hundred eighty days of age. On the check group IV D, the animals came within 0.2 of 1 per cent of equaling the height percentage of the original group. The drop in weight percentage could easily be blamed on the poor

TABLE 2

Average total grain consumed, and increase in weight and height from thirty days to one hundred eighty days of age

	WEIGHT	HEIGHT	GRAIN
	pounds	*cm.*	
I. Holstein 8	196 0	19 5	666 9
I. Ayrshire 3	160 5	18 5	619.6
I. Jersey 2	159 3	16 6	307.3
I. Average	182 7	18 8	645.7
II. Mixed 5	208 4	20.3	734 5
III. Holstein Grade 2	221 5	21.6	852 3
IV. A Holstein Grade 7	234 9	21.1	715 0
IV. B Holstein Grade 4	147.0	17 7	503 2
IV. C Holstein Grade 4	185 2	20 6	597.4
IV. D Holstein Grade 4	197.6	24.1	621.7
V. Holstein Grade 4	212 4	19 9	535.3
VI. Holstein Grade 2	185.5	18 3	521 0

quality of hay consumed and also to the fact that they were limited to almost 100 pounds less grain than the IV A group.

By consulting figure 4, a comparative value of the various mixtures may be worked out. According to that chart, IV A and IV D are the two best groups and both of those are using the same ration.

Animals raised on dry grain mixtures do not seem to have their breeding powers lessened in any way nor do they seem to have their lactating powers diminished. One Holstein two years old made a world's record for both milk and butter in class B. One Ayrshire stands second in the state in production and the others are milking on a par with daughters of the same bulls.

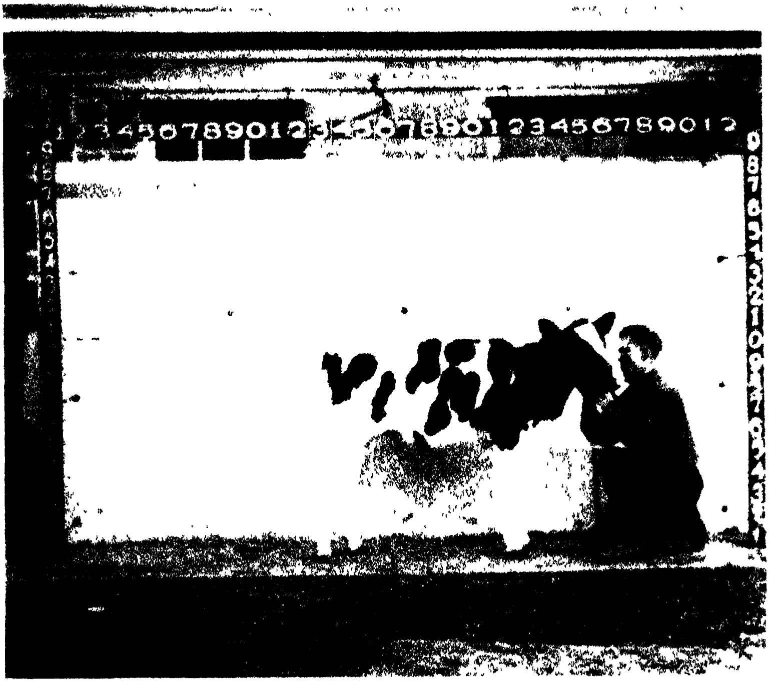
CONCLUSION

Calves can be successfully raised on a dry grain mixture after being weaned from milk at thirty days of age. On a sound dry grain mixture and alfalfa hay fed ad libitum these calves will be 100 per cent normal for weight and height when compared to Eckles normal figures. A dry grain ration will cut the feed costs on raising calves to six months of age from twenty-five to fifty dollars depending on the feeding methods used. This method of feeding saves labor costs and does not harm the breeding or productive ability of the individuals.

REFERENCES

(1) Mead, S. W., Regan, Wm. M., and Bartlett, John W. 1924 A study of the factors affecting the growth of dairy heifers. Jour. Dairy Sci., vii, no. 5.

(2) Eckles, C. H. 1920 The normal growth of dairy cattle. Mo. Agri. Exp. Sta., Res. Bul. 36.



RATION IV D. 158 DAYS WEIGHT 265 POUNDS. HEIGHT 98 CM

Picture illustrating the photographic method of measurement used in all calf feeding work at the New Jersey Agricultural Experiment Station Pictures are taken monthly with animals posed in the same manner each time. The camera is on a rigid support at a fixed distance from the object.

. Horizontal lines appearing on the print to get height point perspective are scratched on the negative.

A STUDY OF THE EFFECTIVENESS OF SODIUM HYPOCHLORITE IN STERILIZING CREAMERY EQUIPMENT*

E. L. FOUTS

Department of Dairying, Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma

Milk centralizing and distributing plants in Oklahoma are required by law to pasteurize all milk intended for retail trade. The purpose of this law is to insure that safe milk of low bacterial content, and of relatively high keeping quality be supplied to the ultimate consumer. However, sanitary conditions in the plant may be such that milk of good quality and low bacterial content may be pasteurized according to law, and yet may be so recontaminated before being placed in the bottles, that the purposes of the law are entirely defeated. The recontamination may arise from such equipment as the sanitary piping, pumps, filters, or coolers with which the milk may come into contact before being bottled. All of these pieces of equipment may appear sufficiently clean to the casual inspector, and still may be important sources of recontamination.

It has been known for years that steam or boiling water were very efficient as sterilizing agents. It has also been recognized that steam must be under pressure in order to accomplish complete sterilization. It is very difficult to sterilize a piece of equipment by simply turning the steam onto it from a steam hose because the surrounding air quickly absorbs the heat from the steam, and not enough heat is applied directly to the organisms present to destroy them. Due to the size of much of the creamery equipment it is impossible to use steam under pressure.

The process of chemical sterilization is not new as it has been applied in other industries for many years. However its use with dairy equipment presents an entirely different problem. Dairy products absorb foreign odors readily, and for this reason,

---

* Received for publication July 18, 1928.

care must be taken in the selection of a suitable chemical. One must be chosen that will kill the organisms present and still impart no flavor or odor to the product. This eliminates many chemicals from use in the milk plant. Many experiments on record show the attempts made to find a suitable material for this purpose.

Sodium hypochlorite was found to kill the bacteria on the utensils if used in the proper manner, with the advantage that it imparted no flavor or odor to dairy products. It has the disadvantage of decomposing rather rapidly when put into solution, letting the active ingredient, chlorine, escape into the air. This disadvantage has largely been overcome by a process used by manufacturers of commercial products for chemical sterilization. The process renders the chlorine partially inert until diluted with water, when ready for use.

Considerable work has been done, using chemicals in cleaning and sterilizing milking machine parts and milk bottles. To the writer's knowledge very little experimental work has been conducted dealing entirely with the sterilization of pumps, pipes and coolers with chemical in an effort to prevent recontamination from these sources after pasteurization. This paper will take up this phase of the work in detail.

REVIEW OF LITERATURE

Fellers and Dearstyne (1) concluded that ". . . . the use of sodium hypochlorite is the equal of steam in the sterilization of milk bottles and dairy utensils." Since sodium hypochlorite is relatively inexpensive, easy to use, and entirely harmless, they strongly recommend its use.

Prucha (2, 3) delivered an address before the International Milk Dealers Association in which he stated that "A chemical that is fit for use with milk must be harmless. Hypochlorites fulfill this condition. Under certain conditions it is advisable to use chemical sterilizers for the sterilization of utensils. The usual simple procedure is to add the chemical to the rinse water and then thoroughly rinse the utensils."

Whittaker and Archibald (4) prepared a report dealing with the sterilization of milk bottles at small milk plants and dairies. In part they wrote: "Experiments were conducted which showed that satisfactory results could be obtained by chemical sterilization, provided the proper quantity of the chemical was used and the bottles completely submerged in the sterilizing solution." In the report a diagram of a simple machine was shown, the purpose of which was to submerge the bottles in a chemical bath, making sure that no air bubbles were in the bottles thereby causing imperfect sterilization. They (5) also showed the relative efficiency of methods of sterilization of milk bottles at pasteurization plants in Minnesota, stating in part that "A study of the results obtained in the sterilization of bottles using steam as compared with chlorine, indicates that chlorine is the more dependable as applied in routine practice. This is especially evident in smaller pasteurization plants where manually operated apparatus is used."

It will be seen from the above references that in all cases chlorine disinfectants are very effective in the destruction of bacteria. Some forms of chlorine disinfectants have the disadvantage of decomposing rapidly when diluted with water, therefore solutions should be prepared as needed.

In the last few years a number of commercial preparations have been placed on the market under various trade names, some in liquid form and others in crystals or granular form.

It was the purpose of this work to study several of these commercial sterilizing agents collectively and to determine their value in the destruction of bacteria on creamery equipment. This experiment dealt with chlorine compounds and their use in sanitary piping, pumps, filters and surface coolers in order to prevent recontamination of pasteurized milk from these sources.

PLAN OF EXPERIMENT

This experiment was conducted in the college creamery under conditions found in the operation of a commercial milk plant. The market milk unit was selected as being suitable for the investigation as it represented typical equipment found in milk plants throughout the state. The milk unit included the following equipment.

200-gallon Cherry Horizontal Coil Pasteurizer
Viking Milk Pump
Sanitary Piping
Chicago Filter
Cherry Cone Cooler (water)
Cherry Tubular Cooler (brine)
Cherry Hand Bottler

The method of handling the milk was as follows: The milk was dumped into the coil pasteurizer through a strainer cloth. It was tested immediately and standardized for fat. The milk was then heated to 145°F. and held for thirty minutes, after which it was cooled to 122°F. by running water through the coils. It was then pumped through the sanitary pipes and filter, over the small conical cooler and then over the large tubular brine cooler, to the bottler.

The investigation was divided into two parts; namely: (*a*) the control part and (*b*) the experimental part. During the control part of the work ordinary washing methods were used in cleaning the equipment. These methods consisted of scrubbing the equipment with a brush, using a hot alkali solution. The entire unit was rinsed with scalding water just before being used.

The experimental part consisted of the identical washing methods described above, followed by a solution of the chlorine sterilizing compound which was prepared in the pasteurizing vat according to the directions of the manufacturers. The sides of the vat and the coil were thoroughly brushed with the solution. The solution was then passed through the pump, pipes, filter and over the two coolers. It was not rinsed from the equipment. After contact with the chlorine solution approximately ninety minutes elapsed before the milk was pumped through the unit.

As a control measure the percentages of active chlorine in the sterilizing solutions were determined by means of the Nafis active chlorine tester.

In order to observe the effect of chlorine sterilization of the equipment upon the bacterial count of the pasteurized milk, samples were taken for bacterial analyses (*a*) from the vat after pasteurization and (*b*) from the trough of the last cooler after the milk had passed through the pump, pipes, filter and over the two coolers.

TABLE 1

Increase in bacterial contents of milk from the market milk unit cleaned by ordinary methods, and from the same unit similarly cleaned and then rinsed with a solution of hypochlorite

TRIAL NUMBER	ORDINARY WASHING METHOD	HYPOCHLORITE NO 1	HYPOCHLORITE NO. 2	HYPOCHLORITE NO 3
1	54,700	3,000	4,000	2,000
2	6,500	1,000	1,000	200
3	32,000	500	1,000	1,500
4	40,000	2,000	1,000	500
5	5,000	1,000	1,000	500
6	12,000	8,000	No increase	800
7	26,000	600	1,500	700
8	12,000	No increase	800	200
9	4,000	400	2,000	1,000
10	3,900	1,300	300	800
11	4,000	1,000	600	3,500
12	5,000	2,000	800	No increase
13	3,300	13,000	800	1,000
14	10,000	No increase	300	800
15	No increase	No increase	No increase	200
16	2,000	500	1,000	
17	4,000	300	10,000	
18	4,000	No increase	3,000	
19	13,500	100	No increase	
20	12,000	500	1,000	
21	7,500	1,000	700	
22	No increase	1,000	500	
23	2,500	1,000	800	
24	4,600	400	600	
25	5,100	500	300	
26	1,500	1,400	1,000	
27	7,300	1,000	200	
28	5,000	1,000	No increase	
29	3,800	1,000	300	
30	1,400	500	200	

Bacterial contents of the samples of milk were determined by the Agar plate method using nutrient agar as the media. One cubic centimeter samples were employed using dilutions of 1:100, 1:1,000 and 1:10,000 from the original milk sample, using sterile water. Data were obtained from these in accordance with standard bacteriological methods. Any increase in bacterial count was attributed to contamination, while passing through and over the several pieces of equipment.

PRESENTATION OF DATA

The data given in table 1, show the results obtained from the experimental work as outlined in the preceding pages. The commercial preparations are considered individually and their germ killing power noted.

The original bacterial counts of the milk before passing through the milk unit following pasteurization, are omitted for brevity. However, of the thirty trials under ordinary washing methods, only two showed an increase of less than 10 per cent in bacterial numbers, while twenty trials showed an increase ranging from 14 to 99 per cent, and eight trials showed an increase of over 100 per cent. The average increase was 74.8 per cent in bacterial numbers.

When the hypochlorite solutions were used to rinse the equipment after it was cleaned by ordinary washing methods, about ninety minutes elapsed between the time of rinsing and contact of the unit with milk. After using hypochlorite solution No. 1, twenty-five trials showed an increase of less than 10 per cent in bacterial numbers, the average being a 10.4 per cent increase. When hypochlorite solution No. 2 was used as the rinse, twenty-seven of the thirty trials showed an increase of less than 10 per cent in bacterial numbers, and only three above this percentage, the average being an 8 per cent increase. Of the fifteen trials when hypochlorite solution No. 3 was used as the rinse, fourteen showed less than a 10 per cent increase. The average of trials when this solution was used, showed only 9.8 per cent increase in bacterial due to contamination from the milk unit, or from the breaking up of clumps of bacteria by agitation of the milk.

It will be seen that in a large percentage of these trials, the pasteurized milk sustained an increase of less than 10 per cent in bacterial numbers while passing through the chemically treated equipment. Such a control of bacteria in the equipment is considered quite efficient.

It will be noted in comparing the results given in table 2, obtained by ordinary washing methods, and those with additional use of a commercial hypochlorite solution, in sterilizing milk plant equipment, that over 85 per cent of contamination of pasteurized milk was prevented.

TABLE 2

Comparison of ordinary washing methods with the results obtained by an additional chemical rinse with commercial sodium hypochlorite solutions

NUMBER OF TRIALS	HYPOCHLORITE SOLUTION USED	AVERAGE BACTERIAL COUNT AFTER PASTEURIZATION	AVERAGE BACTERIAL COUNT AFTER PASSING THROUGH EQUIPMENT	AVERAGE INCREASE
				per cent
30	None	12,560	21,913	74 8
30	1	12,300	13,583	10 4
30	2	11,866	12,823	8 0
15	3	9,086	9,986	9 8

TABLE 3

Active chlorine in the rinsing solutions prepared from commercial hypochlorite solutions used

HYPOCHLORITE	NUMBER OF DETERMINATIONS	PARTS OF ACTIVE CHLORINE PER MILLION	
		Range	Average
1	15	45–50	48
2	15	47–52	50
3	8	50–54	51

An indication of the strength of solutions used in this experiment, as determined by the Nafis Active Chlorine Tester, is shown in table 3. The solutions were checked for active chlorine in half of the trials to make certain that the proper chlorine strength was being obtained in the rinsing solution. The extreme range in concentration of active chlorine used, was found to be 45 to 54 parts per million.

It may be seen that the solutions prepared in a definite manner as directed by the manufacturer, made from fresh chemical, will yield a rinse of practically constant chlorine content.

DISCUSSION OF RESULTS

Although little work has been done on exactly the same problem studied here, these results agree closely with those obtained by other investigators. Any differences that occur between the results of this work and others, may possibly be attributed to the work being carried on under different conditions. It will be noticed that the bacterial counts are relatively high in this investigation as compared with work done by others. This may be due to the work being conducted during the hot weather of the late summer when the raw milk received was very high in bacterial content. However, the results are comparative and serve to show the effect of chlorine sterilizing agents.

For the benefit of those who might wish to make use of the results obtained, it may be said that the dilutions of the chemical sterilizing agents were prepared exactly as recommended by the manufacturer of each product.

The strength of the solutions used, as checked by means of the Nafis Active Chlorine Tester, were found to range between 45 and 54 parts of active chlorine per million parts of solution.

It was seen that ordinary washing methods do not accomplish sterilization. Milk passing through equipment cleaned by these methods, is heavily recontaminated. The additional use of commercial hypochlorite solutions for rinsing the milk plant equipment was found in the majority of trials to minimize the increase in bacterial content of pasteurized milk due to contact with the equipment. In 86 to 97 per cent of the trials observed, the increase in bacterial count was less than ten per cent, perhaps partly due to breaking up of clumps of bacteria by agitation of the milk.

CONCLUSIONS

1. Ordinary washing methods followed by a rinse of scalding water do not sterilize dairy equipment.
2. Sodium hypochlorite is effective as a sterilizing agent for dairy equipment when used in the proper strength.
3. The sodium hypochlorite products used, were effective when

prepared according to the directions furnished by the manufacturers, and providing a minimum of 45 parts of active chlorine per million parts of solution.

4. The bacterial count of milk after pasteurization can be held to a 10 per cent increase, from the pasteurizer to the bottle by the use of sodium hypochlorite in sterilizing the equipment.

The author wishes to acknowledge sincere appreciation to Professor A. C. Baer, Head of Dairy Department, Oklahoma Agricultural and Mechanical College, for assistance in formulating plans for this experiment, and to Dr. R. B. Becker, Oklahoma Agricultural and Mechanical College, for helpful suggestions and constructive criticism of the manuscript.

REFERENCES

(1) Fellers, C. R., and Dearstyne, R. S. 1925 Efficiency of various methods of washing and sterilizing dairy utensils. Jour. Dairy Sci., viii, 163–166.

(2) Prucha, M. J. 1926 Chemical Sterilization of Dairy Equipment. Illinois Sta. Rpt., 93–97.

(3) Prucha, M. J. 1927 Chemical Sterilization in the Dairy Industry. Address before International Association of Dairy Milk Inspectors, Toronto, Ontario.

(4) Whittaker, H. A., and Archibald, R. W. 1924 An Apparatus for Sterilizing Milk Bottles at Small Milk Plants and Dairies. Minn. State Bd. of Health, Division of Sanitation, pamphlet.

(5) Whittaker, H. A. 1924 Relative Efficiency of Methods of Sterilization of Milk Bottles at Pasteurization Plants in Minn. Reprint 918, Pub. Health Reports, Minn. State Bd. of Health, Division of Sanitation.

THE RELATION BETWEEN WEIGHT AND FAT PRODUCTION OF GUERNSEY CATTLE*

CHARLES W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia, Missouri

A study of the relation between weight and fat production of Register of Merit Jersey cows has already been reported (1, 2). It was shown that within the weight range included there was an increase of 104 pounds in fat production per year for an increase of 100 pounds of body weight. However, when the age was made constant, there was an increase of only 20 pounds of fat for each 100 pounds of body weight. From this it was concluded that an increase of body weight contributes about 20 per cent to the total increased fat yield with age while the other 80 per cent of increased fat yield with age was due to other factors accompanying increased maturity.

Through the coöperation of Guernsey breeders, the American Guernsey Cattle Club has obtained body weights of more than 2700 animals with Advanced Register records. These data include both actual and estimated body weights at the completion of official records. A short report of these data has been published (3). While the number of records is limited it was believed to be a representative population of such animals. It was, therefore, thought desirable to make a study similar to that reported on the Jerseys. Through the kindness of Mr. C. M. Cummings of the Advanced Registry Division of the American Guernsey Cattle Club, the writer was furnished a copy of the original data.

For purposes of comparison with similar data already reported, it should be noted that the age for the Jerseys was reported at the commencement of the test, whereas in this case the age was taken at the close of the test. Thus while the weights for both breeds were taken at the close of the test, in the case of the Jersey, the age at the beginning of test was given while in the

---

* Received for publication July 24, 1928.

case of the Guernsey, the age is given at the close of the test. The three- to four-year-old cows in this study are comparable with the two- to three-year-old Jersey cows.

ANATOMICAL RELATIONS

The secretion of milk and fat by the dairy cow is regulated by many factors. The mass of secretory tissue in the udder is of utmost importance. While it may be possible for the rate of secretion by a given mass of secretory tissue to vary to some degree, there are probably fairly definite upper limits in this regard. Thus the final limiting factor in regard to the total milk and fat produced in a stated period of time must be the amount of secretory tissue, the amount of storage space in the udder, and the frequency of removal of milk.

A second factor concerned with milk secretion is the volume of blood passing to the mammary glands. In thin cattle Trowbridge, Moulton, and Haigh (4, 5) found that about 5 per cent of the live weight consisted of blood. While the blood supply may, therefore, be considered to bear a fairly definite relation to live weight, it does not necessarily mean that it is always made available to the mammary glands in the same proportion. The vascular system of the udder is on a branch which periodically fluctuates greatly in respect to the volume of blood passing through it.

A third factor which should be mentioned is the ability of the cow to consume and digest large quantities of feed. Until accurate data are at hand, it seems logical to assume that there is a relation between size (body weight) and this ability.

In conclusion it may be noted that when a large body is associated with small mammary glands, the usefulness of the animal as an economical producer of milk and fat would be limited by the amount of secretory tissue and storage space for the milk. When a small body is associated with large mammary glands (assuming a large amount of secretory tissue and storage space for the milk) it is probable that the supply of precursors of milk in the blood is the limiting factor. The cows in this latter class would more nearly show the importance of live weight in connection with milk secretion, whereas the former would not. These differences should be considered in studying the results.

LIVE WEIGHT AND YEARLY FAT PRODUCTIONS

It has been shown (1) in the case of the Jersey cow that from the age when milk secretion usually begins (two years) to the age when maximum body weight is reached (about eight years), increase of yearly fat production and increase of body weight with age follow the same exponential course. The same was true of the change in fat production with age in the Guernsey breed.

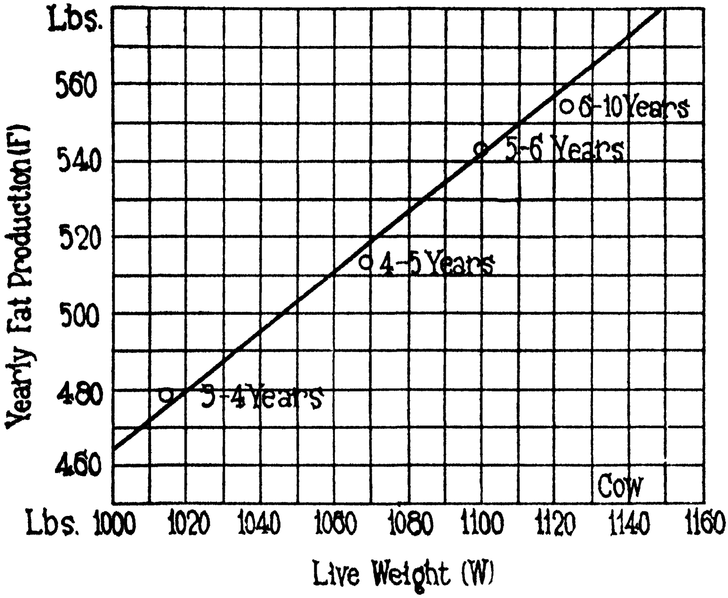


Fig. 1. The Increase of Yearly Fat Secretion with Increase in Age and Live Weight in Guernsey Cattle

The line passing through the observed values was fitted by the method of least squares. With the determinations of the parameters, the equation takes the form $F = 0.77W - 304.73$ in which F is the yearly fat production for any live weight W. This equation indicates that with an increase of 100 pounds in live weight accompaning age there is an increase of 77 pounds of fat per year above 304.73 pounds. The corresponding equation for the Jersey breed was $F = 1.042W - 472.32$.

TABLE 1

Relation between live weight and yearly fat production (Guernsey cattle)

AGE CLASS	NUMBER OF COWS INCLUDED	LIVE WEIGHT			YEARLY FAT PRODUCTION			COEFFICIENT OF CORRELATION
		Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation	
years		*pounds*			*pounds*			r
3 to 4	1,149	1,014.9 ±2 31	116.6 ±1.63	11 49 ±0 16	478 6 ±1 75	88 35 ±1.24	18.46 ±0.27	252 ±0.019
4 to 5	508	1,068.7 ±3.75	125 25 ±2 65	11 72 ±0 25	513 8 ±2 93	97.95 ±2 07	19.06 ±0 42	256 ±0 028
5 to 6	352	1,100.1 ±4 58	127.35 ±3.23	11.56 ±0 32	543 3 ±3 64	101.44 ±2 58	18 67 ±0 49	336 ±0 032
6 to 10	500	1,113 8 ±3 86	128.00 ±2.73	11.49 ±0 25	554 5 ±3 11	103 1 ±2 20	18.59 ±0 41	243 ±0.028

Class interval. Weight 50 pounds, fat production 25 pounds. Weighed average r = 0.263.

Recently (6) the writer presented data indicating that the course of growth in weight of Guernsey cows could also be represented by an exponential equation. The similarity of the two equations is apparent.

$$\text{Weight} = 1130\ (1 - e^{-0.55t})$$
$$\text{Yearly fat production} = 532\ (1 - e^{-0.58t})$$

In this equation e is the base of natural logarithms, and t is the age. As growth in weight and increase in yearly fat production follow the same course with age there should be a directly proportional relation between the two factors. That this is indeed true is shown by figure 1 which has been plotted from the data in table 1. The line passing through the points was fitted by the method of least squares. The equation obtained was of the form

$$F = 0.77W - 304.73$$

in which F is the yearly fat production for any live weight W. This equation indicates that with an increase of 100 pounds in live weight accompanying age there is an increase of 77 pounds of fat per year above 304.73 pounds.

The corresponding equation for the Jersey breed was

$$F = 1.042W - 472.32$$

These equations indicate first that on the average there is a greater increase in yearly fat production for 100 pounds increase in live weight in the Jersey than in the Guernsey breed. The equation further indicates a possible theoretical weight at which this relation starts. If the relation extends below the observed weights, it would mean that milk secretion should be initiated in Guernsey cattle when they reach about 300 pounds, whereas the corresponding weight for Jersey heifers to be able to start milk secretion should be about 470 pounds. While milk secretion can be induced in heifers with recurring oestrous cycles, the quantity is very limited until well along in pregnancy. Until further data are at hand, it seems better to suspend judgment in regard to this point and apply the equation only within the weight range observed.

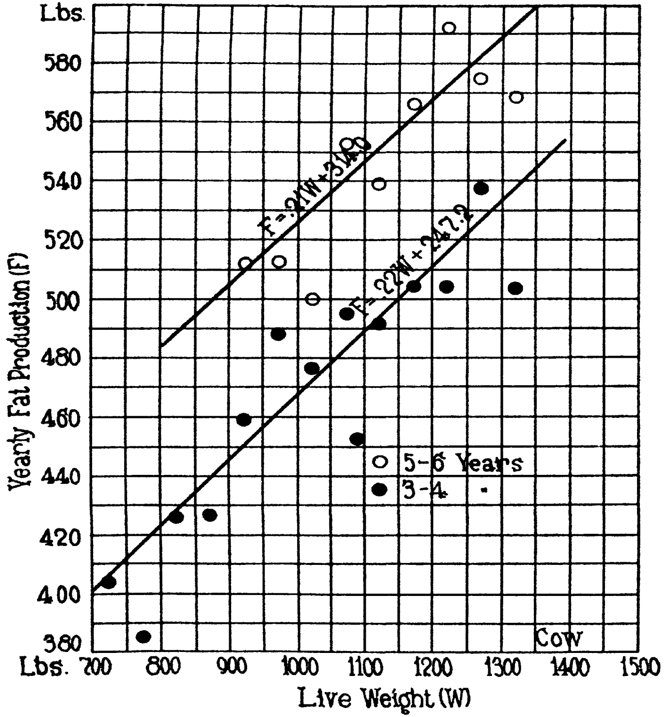


Fig. 2

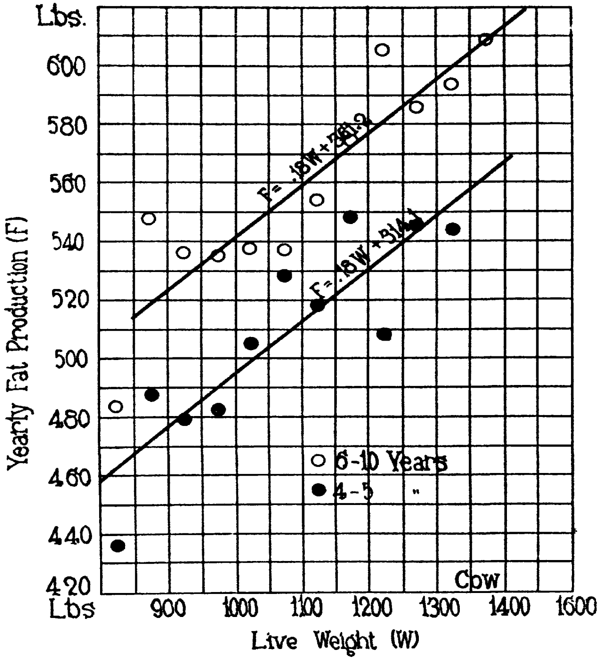


Fig. 3

Figs 2 and 3. Presenting the Increase in Fat Secretion with Increasing Body Weight for Various Age Groups (Age Held Constant)

The straight lines passing through the observed values were fitted by the method of least squares from the equation of the form $F = aW + b$ in which F is the yearly fat production for the body weight W; a is the constant increase of yearly fat production for each added pound of body weight. From the values of a indicated on the lines, it will be noted that an increase of 100 pounds in the weight of the body is accompanied by an increase, on the average, of 20 pounds of fat production per year.

THE INFLUENCE OF WEIGHT ON FAT PRODUCTION AT CONSTANT AGE

The study of the relation between weight and fat production considers only two of the three factors concerned in the problem. The factor of age also requires study. As age increases, there are recurring pregnancies which cause further growth of the mammary gland epithelial secreting tissue. With age also there is increase in live weight. To more accurately determine the effect of live weight alone on yearly fat secretion, the relation between these two variables should be studied *holding age constant.*

TABLE 2

Relation between weight and fat production at constant age

Age, 3 to 4 years

BODY WEIGHT MID-CLASS VALUE	NUMBER OF COWS INCLUDED	YEARLY FAT PRODUCTION CALCULATED FROM EQUATION*	MEAN YEARLY FAT PRODUCTION	STANDARD DEVIATION	COEFFICIENT OF VARIATION
pounds		*pounds*	*pounds*		
725	12	406.7	404.5 ±13.35	68.50 ± 9.41	16.93 ±2.25
775	19	417.7	385.2 ±10.80	69.53 ± 7.60	18.05 ±2.03
825	61	428.7	426.1 ± 6.48	75.00 ± 4.58	17.61 ±1.06
875	81	439.7	426.7 ± 7.04	94.00 ± 4.98	22.26 ±1.22
925	166	450.7	459.0 ± 4.40	83.25 ± 3.08	18.13 ±0.69
975	193	461.7	488.2 ± 4.10	84.50 ± 2.90	17.31 ±0.59
1,025	196	472.7	475.8 ± 3.52	84.25 ± 2.87	17.71 ±0.60
1,075	157	483.7	494.5 ± 4.75	88.25 ± 3.36	17.84 ±0.67
1,125	141	494.7	491.8 ± 4.39	77.25 ± 3.10	15.71 ±0.62
1,175	51	505.7	504.3 ± 8.19	86.75 ± 5.79	17.21 ±1.17
1,225	44	516.7	504.0 ± 8.74	86.00 ± 6.18	17.06 ±1.26
1,275	12	527.7	537.5 ±16.84	86.50 ±11.91	16.09 ±2.25
1,325	11	538.7	503.5 ±15.13	74.25 ±10.68	14.74 ±2.05
1,375	4	549.7	556.0		
1,425	1	560.7	512.5		
Total...	1,149				

* Equation fitted by the method of least squares $F = 247.2 + 0.22W$ where F is the yearly fat production (pounds) and W is live weight at the close of the lactation.

In the two year old group, for example, it is practically certain that the mammary glands of all the animals included have only been developed by a single pregnancy. Differences in production within the group may be largely ascribed to differences in live weight, although other factors may not be entirely ruled out.

If, then, comparisons are made between two year old cows and three year old cows at a constant weight, say 1100 pounds, the differences may be ascribed chiefly to differences accompanying age aside from weight. The increased production is thought to be a measure of the effect of a second pregnancy in stimulating further growth of mammary gland tissue.

TABLE 3

Relation between weight and fat production at constant age

Age, 4 to 5 years

BODY WEIGHT MID-CLASS VALUE	NUMBER OF COWS INCLUDED	YEARLY FAT PRODUCTION CALCULATED FROM EQUATION*	MEAN YEARLY FAT PRODUCTION	STANDARD DEVIATION	COEFFICIENT OF VARIATION
pounds		*pounds*	*pounds*		
725	5	444.6	507.0		
775	3	453.6	579.2		
825	10	462.6	436.0 ±16.37	76.75 ±11.58	17.60 ±2.64
875	14	471.6	487.5 ±18.25	101.25 ±12.75	20.79 ±2.65
925	55	480.6	478.9 ± 8.00	88.00 ± 5.66	17.67 ±1.12
975	60	489.6	482.5 ± 6.90	79.25 ± 4.88	18.03 ±1.14
1,025	87	498.6	505.6 ± 6.98	96.50 ± 4.93	16.42 ±0.84
1,075	77	507.6	528.5 ± 7.84	102.00 ± 5.55	20.85 ±1.13
1,125	74	516.6	518.9 ± 6.96	88.75 ± 4.92	17.29 ±0.97
1,175	47	525.6	548.1 ± 8.56	87.00 ± 6.05	15.87 ±1.07
1,225	36	534.6	508.5 ±11.52	102.50 ± 8.15	22.01 ±1.83
1,275	21	543.6	545.9 ±15.57	105.75 ±11.01	19.37 ±2.05
1,325	13	552.6	543.3 ±22.45	120.00 ±15.87	22.08 ±3.17
1,375	2	561.6	470.0		
1,425	3	570.6	587.5		
1,475	0	579.6			
1,525	1	588.6	662.5		
Total...	508				

* Equation fitted by the method of least squares $F = 314.1 + 0.18W$ where F is the yearly fat production (pounds) and W is live weight at the close of the lactation.

The results of the tabulation are presented in tables 2 to 5 inclusive, and figures 2 and 3. The lines passing through the observed points were fitted by the method of least squares.

The equations obtained were as follows:

Age 3 to 4 years $F = 0.22W + 247.2$
Age 4 to 5 years $F = 0.18W + 314.1$
Age 5 to 6 years $F = 0.21W + 314.9$
Age 6 to 10 years $F = 0.18W + 361.2$

where F is the yearly fat production for any live weight W. These equations indicate that at a constant age there is an increase of from 18 to 22 pounds yearly fat production for an increase of 100 pounds of weight.

TABLE 4

Relation between weight and fat production at constant age

Age, 5 to 6 years

BODY WEIGHT MID-CLASS VALUE	NUMBER OF COWS INCLUDED	YEARLY FAT PRODUCTION CALCULATED FROM EQUATION*	MEAN YEARLY FAT PRODUCTION	STANDARD DEVIATION	COEFFICIENT OF VARIATION
pounds		*pounds*	*pounds*		
725	2	467.2	412.5		
775	3	477.7	479.2		
825	4	488.2	481.3		
875	7	498.7	508.9		
925	28	509.2	511.6 ± 9 45	74.25 ± 6.69	14 51 ±1.29
975	26	519.7	512.5 ±10.05	75.96 ± 7.10	14 82 ±1.33
1,025	59	530.2	499.4 ± 8.64	98 44 ± 6.19	19.71 ±1.22
1,075	44	540.7	552 8 ± 8 99	88 50 ± 6 36	16 08 ±1.17
1,125	57	551.2	539 3 ± 8.88	99.34 ± 6.28	18 42 ±1.17
1,175	42	561.7	566.1 ±10.69	100 68 ± 7 57	17.78 ±1.29
1,225	44	572.2	592 6 ± 9 51	93.48 ± 6 72	16.20 ±1.18
1,275	19	582.7	575.7 ±14.44	93 32 ±10 21	16.20 ±1.80
1,325	8	593.2	568.8 ±29.17	122.31 ±20.62	22.15 ±3 88
1,375	4	603.7	625.0		
1,425	4	614.2	662.5		
1,475	0	624.7			
1,525	1	635 2	687.5		
Total...	352				

* Equation fitted by the method of least squares $F = 314.9 + 0.21W$ where F is the yearly fat production (pounds) and W is live weight at the close of the lactation.

In this respect, the Jersey data were quite similar in that the average increase in fat production was about 20 pounds for each 100 pounds increase in live weight.

This finding, as already pointed out (1) is of considerable economic importance in that at ordinary prices for butterfat, 20 pounds is barely sufficient to cover the cost of maintenance of 100 pounds of body weight per year.

Returning again to the question of the relative importance of weight at a constant age and the other factors accompanying age, such as recurring pregnancies which may have an influence on yearly fat production. From figure 1, it will be noted that the total increase in fat production was 77 pounds per 100 pounds increase in live weight, while in figures 2 and 3 at constant age, the average increase in fat production is only 20 pounds per 100

TABLE 5

Relation between weight and fat production at constant age

Age, 6 to 10 years

BODY WEIGHT MID-CLASS VALUE	NUMBER OF COWS INCLUDED	YEARLY FAT PRODUCTION CALCULATED FROM EQUATION*	MEAN YEARLY FAT PRODUCTION	STANDARD DEVIATION	COEFFICIENT OF VARIATION
pounds		*pounds*	*pounds*		
725	1	491.7	462.5		
775	1	500.7	437.5		
825	8	509.7	484.1 ±22.98	96.37 ±16.25	19.91 ±3.32
875	10	518.7	547.5 ±21.22	99.50 ±15.01	18.17 ±2.802
925	29	527.7	536.6 ±10.44	83.32 ± 7.38	15.53 ±1.36
975	47	536.7	535.9 ± 8.50	86.51 ± 6.02	14.37 ±0.99
1,025	63	545.7	537.9 ± 7.72	90.80 ± 8.09	16.88 ±0.99
1,075	61	554.7	537.1 ± 8.35	96.65 ± 8.79	17.99 ±1.07
1,125	92	563.7	554.3 ± 6.84	97.24 ± 4.84	17.54 ±0.87
1,175	60	572.7	574.2 ± 8.12	93.26 ± 5.74	16.24 ±1.01
1,225	65	581.7	605.6 ± 9.09	108.62 ± 6.43	17.94 ±1.03
1,275	28	590.7	585.9 ±14.07	110.40 ± 9.95	18.84 ±1.67
1,325	18	599.7	594.4 ±20.47	128.78 ±14.48	21.66 ±2.37
1,375	9	608.7	609.7 ±23.01	102.36 ±16.27	16.79 ±2.61
1,425	4	617.7	562.4 ±38.35	113.71 ±27.12	20.22 ±4.95
1,475	2	626.7	437.5		
1,525	2	635.7	525.0		
Total...	500				

* Equation fitted by the method of least squares $F = 361.2 + 0.18W$ where F is the yearly fat production (pounds) and W is live weight at the close of the lactation.

pounds increase in live weight. From these figures it would appear that about 25 per cent ($\frac{20}{77}$) of the total increase of fat secretion with age is due to the increase of the live weight of the animals concerned. The other 75 per cent of the increase in fat secretion with age would be ascribed to the development of the udder by recurring pregnancies, and other factors which might cause the glands to become more efficient.

If we may assume that on the average, each successive yearly age class represents the recurrence of a pregnancy, a rough measure of the actual gland growth may be obtained, up until maturity is reached, when it is probable that further growth does not equal the loss due to the destruction and death of epithelial cells.

TABLE 6

Increase in fat production due to weight changes and development of the mammary glands with age

AGE	YEARLY FAT PRODUCTION (CALCULATED)	INCREASE IN FAT PRODUCTION OVER PRECEDING YEAR	INCREASE IN FAT PRODUCTION DUE TO WEIGHT CHANGE	INCREASE IN FAT PRODUCTION DUE TO DEVELOPMENT OF GLANDS
years	*pounds*	*pounds*	*pounds*	*pounds*
2 5	410			
3.5	461	51	13 0	38.0
4.5	491	30	7.5	22 5
5.5	511	20	5 0	15 0
6.5	521	10	2 5	7.5
7.5	528	7	2.0	5.0
		118	30.0	88.0

The data in table 6 indicate that the development of the mammary glands during the first pregnancy is very great. Hammond (7) figures a series of udders which readily demonstrate the really tremendous growth and development which takes place during the first pregnancy in heifers. The total increase in fat production above the two years old production is 118 pounds of fat, or 28.8 per cent. Of this, 88 pounds of fat or 21.5 per cent is thought to be due to changes with age (chiefly recurring pregnancies) aside from increase in live weight, and only 30 pounds or 7.3 per cent of the increase in fat due to weight.

The table shows further that the second pregnancy causes only a relatively slight increase in secreting tissue (sufficient to secrete 38 extra pounds of butter fat) and a further decline with each succeeding pregnancy up to maturity.

From a practical standpoint it would appear that the most rapid gland development and the most economical milk and fat production would result from early breeding and a rapid succession of pregnancies. While this plan would not result in the greatest maximum yield during a single lactation, it is believed that it would result in the most economical life time production. Has not too much emphasis been placed upon the development of maximum size if the returns from the greater body weight barely pay for the increased cost of maintenance?

TABLE 7

Comparison of the correlation coefficient and correlation ratio

FACTORS	COEFFICIENT OF CORRELATION r	RATIO OF CORRELATION η	$\eta^2 - r^2$
Age-weight	0 316 ±0 035	0 325	0 006
Age-fat	0 325 ±0 040	0 336	0 007
Weight-fat	0 326 ±0 040	0 336	0 007

Class interval: age 2.5 to 7 years inclusive by 0.5 years, weight 50 pounds, and fat production 25 pounds.

THE CORRELATION BETWEEN AGE, WEIGHT AND FAT PRODUCTION

To further check the accuracy of the observations just presented, it was thought desirable to apply the method of partial correlation to the same data. It is a method which is especially suited to the problem at hand; that is, of determining the relation between two variables while holding the third variable constant.

The following simple correlations were first computed, age-weight, age-fat, and weight-fat covering the age interval between two and one-half to seven years. As is well known, the coefficient of correlation is only adapted for variables which are linearly related. As age-weight and age-fat have both been shown to bear an exponential relationship, justification for the application of the correlation method is required. It will be noted from an inspection of the curves describing these relationships that over a considerable part of the curve a straight line would fit almost as well as the exponential curve. Thus, while this part could not be said to be linear, the degree of departure would not be great.

A further test of linearity is the comparison of the correlation coefficient (r) with the correlation ratio (η). The two values will be identical when the regression is strictly linear, and will differ the more widely the greater the departure from linear form. The general test for linearity is (Zeta) $= \eta^2 - r^2$.

From table 7 it may be seen that over the age range included, no great error is involved in using the coefficient of correlation.

THE PARTIAL CORRELATION

The usual equation of partial correlation is as follows:

$$r_{12.3} \quad \frac{r_{12} - r_{13} \cdot r_{23}}{\sqrt{1 - r^2_{13}}\sqrt{1 - r^2_{23}}}$$

Substituting the simple coefficients of correlation obtained above in this equation, the following partial correlations were obtained:

Partial correlation of age, weight and fat production

FACTORS CORRELATED	FACTOR HELD CONSTANT	PARTIAL R
Age-weight	Fat	0.235
Age-fat	Weight	0.248
Weight-fat	Age	0.249

The partial correlation of weight and fat with age held constant is 0.249. This is slightly lower than the weighted average of the simple correlations for each age group shown in table 1 (0.263). The similarity of this correlation to the figure of 25 per cent ascribed to the influence of live weight on yearly fat production is taken as further confirmation of the accuracy of this relation.

SUMMARY

A study is reported showing the relation between age, live weight, and yearly fat production of over 2700 Guernsey cows.

As increase in yearly fat production and increase of body weight with age follow the same exponential course, it was shown that there was a direct relation between weight and fat production which could be represented by an equation of the first degree.

$$\text{Yearly fat production} = 0.77 \text{ weight} - 304.73$$

This equation indicates that an increase of 100 pounds in live weight accompanying age there is an increase of 77 pounds of fat per year.

However, when age was held constant there was an increase of only 20 pounds of fat for an increase of 100 pounds in weight.

From these figures it is concluded that about 25 per cent ($\frac{20}{77}$) of the total increase of fat secretion with age is due to the live weight of the animals concerned, whereas the other 75 per cent of the increase in fat secretion with age would be ascribed to the development of the udder by recurring pregnancies.

The method of partial correlation was also applied. It was found that the partial correlation of weight and fat with age held constant was 0.249. The similarity of this correlation to the figure of 25 per cent ascribed to the influence of live weight on yearly fat production is taken as further confirmation of this relationship.

REFERENCES

(1) Brody, S., Ragsdale, A. C., and Turner, C. W. 1923 The rate of growth of the dairy cow. III. The relation between growth in weight and increase of milk secretion with age. Jour. Gen. Physiol., vi, no. 1, pp. 21–30.

(2) Turner, C. W., Ragsdale, A. C., and Brody, S. 1924 The relation between age, weight and fat production in dairy cows. Missouri Agr. Exp. Sta. Bul. 221.

(3) Age-weight-fat. What relationship has age and weight to production? Guernsey Breeders' Journal, xxx, no. 8, October 15, 1926.

(4) Trowbridge, P. F., Moulton, C. R., and Haigh, L. D. 1915 The maintenance requirement of cattle. Mo. Res. Bul. 18.

(5) Moulton, C. R., Trowbridge, P. F., and Haigh, L. D. 1922 Studies in animal nutrition. II. Changes in proportions of carcass and offal on different planes of nutrition. Mo. Res. Bul. 54.

(6) Turner, C. W. 1928 Growth in weight of Guernsey cows after the age of two years. Jour. Dairy Sci., xi, no. 3.

(7) Hammond, John 1927 The Physiology of Reproduction in the Cow. Cambridge University Press.

STUDIES ON THE NUTRITIVE VALUE OF MILK

I. THE PRODUCTION OF NUTRITIONAL ANEMIA IN ALBINO RATS THROUGH EXCLUSIVE WHOLE MILK DIETS*

W. E. KRAUSS

Department of Dairying, Ohio Agricultural Experiment Station, Wooster, Ohio

As one of the steps in a study of the food value of milk as affected by various rations, rats were fed exclusive whole milk diets. It was thought that a more accurate measure of the total nutritive effect, aside from reproduction, of milk from various sources could be obtained in this way, inasmuch as no foreign material whatsoever would be introduced. Furthermore, any influence that a basal ration might exert on the inherent properties of fluid milk would be eliminated. The work of Mattill and co-workers (1) (2) (3), Daniels and Hutton (4), Mitchell and Schmidt (5), and Scott (6) showed that reproduction in rats was very unsatisfactory on diets consisting entirely of milk, milk and bread or milk and rice, young rarely being produced beyond the second generation, and that such young were extremely anemic. Consequently the study was confined to growth and physical condition and no attempt was made to add anything to the milk diet that might overcome reproductive troubles. It was expected, owing to the low iron content of such a diet, that anemia would be encountered, but not until sufficient data had been obtained to measure the milk's value for growth.

PRELIMINARY WORK

Nothing but milk from three groups of Holstein cows on widely different rations was fed to three respective groups of rats. With two exceptions, both in the same group, all the rats grew well for at least three weeks. Growth then slowed up, ceasing altogether after six weeks. The rats then maintained their weights for several more weeks, after which there was a rapid decline and

---

* Received for publication August 4, 1928.

death. (In one group all the rats were dead at the end of four weeks. It is believed that some other factor, aside from the main one now under consideration, was responsible for this.) From the appearance of the rats in the period of decline it was assumed that severe anemia was present. This belief was reported in a paper read before the American Dairy Science Association in June, 1927. The eyes were very light pink with a yellowish tinge which gave the appearance of glassiness. The ears, tail and feet were colorless. Post mortem examination confirmed the external symptoms. All the internal organs were very pale, particularly the liver. In all cases the spleen was pathological to a greater or less extent. There was also considerable evidence of intestinal hemorrhage. These findings are similar to those reported by Brouwer (7), who fed exclusive milk diets to rabbits.

Thinking that the nature of the ration fed the cows was a contributing factor in this work, another group of rats was fed nothing but mixed milk from the entire Station herd of Holsteins and Jerseys. The results were practically the same. Evidently milk from a general source was also unable in itself to support growth or prevent death.

Hemoglobin determinations made at this point, August, 1927, showed that the blood of rats that had been on exclusive milk diets for eleven weeks contained between 2 and 3 grams of hemoglobin per 100 cc., whereas that of normal rats from the stock colony contained from 15 to 16.5 grams per 100 cc. This proved beyond question that a severe anemia existed and suggested that this was a limiting factor in growth studies on exclusive milk diets. It also presented the possibility of obtaining anemic rats quickly without resorting to special breeding on diets low in iron, as suggested by other workers.

In order to attain the ultimate object set it became necessary to eliminate the factor of anemia through supplementary feeding without materially affecting the original chemical or physical properties of the milk diet. Using the procedure described below, the effect on nutritional anemia of a large number of substances, including the usual constituents of basal rations and various iron salts, was studied. While the results of this work will be presented in a subsequent paper, it will be of interest to mention that of all the substances tried, the only real success in combating the disease was attained through the use of a specially prepared yeast, hog liver, or solutions of iron and copper fed simultaneously.

PROCEDURE

Weanling rats twenty-four to twenty-eight days of age (weighing from 48 to 52 grams as a rule) were moved to cages provided with screen bottoms. At first four rats, two males and two females, were placed in each cage. This was satisfactory when milk ad lib. was fed and it was desired to produce anemia, or for preliminary work on supplementary feeding, but when accurate additions to the milk were made segregation as individuals was essential. As the object of this study was to prevent anemia from developing, the substance to be tested was added from the beginning in most cases. Control groups received no addenda, except in some cases after severe anemia had developed when it was desired to test the corrective possibilities of some substance. In such cases the hemoglobin content was allowed to drop to between 1.38 and 2.07 grams per 100 cc. of blood before adding the substance under consideration. This afforded a very rigid test of hemoglobin-forming power. After the animals were six weeks of age hemoglobin determinations were made weekly on a different representative of each group. A small piece was cut off the end of the tail with a razor and a freely-flowing drop of blood was transferred by capillarity to the pipette of a Dare hemoglobinometer in which the percentage of hemoglobin was determined. The blood flow was stopped by applying a solution of silver nitrate and alum powder. No difficulty in obtaining a suitable sample was encountered. The rat was held by an assistant while the operator cut the tail, made the blood transfer and read the hemoglobinometer. Three readings were made of each sample, the average being taken for record. It was found unnecessary to apply warm water to the tails to stimulate blood flow. In very stubborn cases gentle stroking of the tail produced the desired result.

The milk was fed in glass beakers and the amount consumed estimated as accurately as possible. It was found that when the appetite was not impaired the per capita daily consumption at two months of age was about 50 cc. When continued at this level good growth was possible provided the milk was suitably supplemented. Milk was fed twice a day, the supplement being added to the afternoon feeding (six times a week), at which time a small enough quantity was fed to insure complete consumption. In order to eliminate the factors of breed and environment the milk used was from Holstein cows kept under winter feeding conditions constantly and receiving a normal dairy ration of alfalfa hay, corn silage, corn, oats, bran and oilmeal.

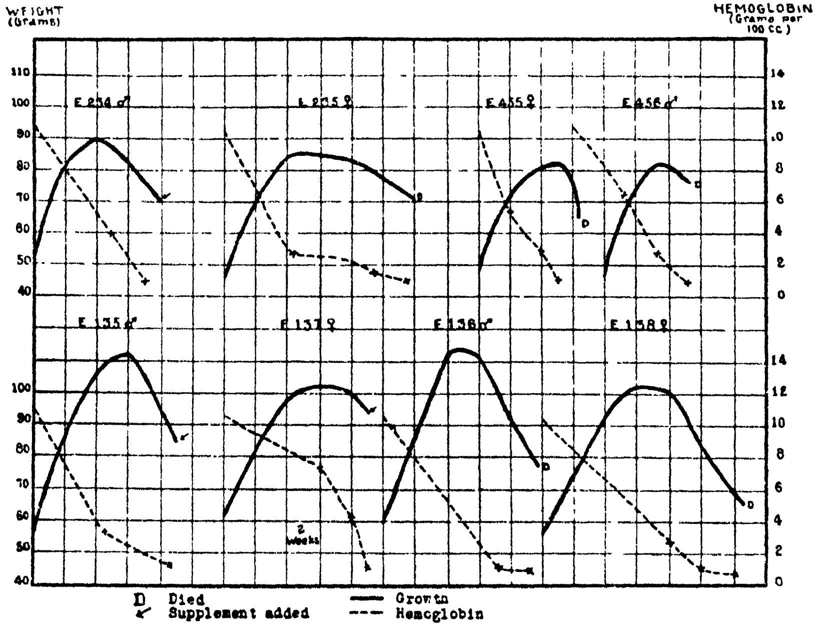


FIG. 1. SHOWING GROWTH CURVES AND HEMOGLOBIN VALUES ON AN EXCLUSIVE WHOLE MILK DIET

The first point on the hemoglobin curves in each case represents the average value for weanling rats from normal females.

While the number of determinations made was quite small, there was no indication that our weanling rats were anemic. However, their hemoglobin index (from 10.33 to 11.02) was lower than it becomes after a very short time on the stock ration[1] (from 15.15 to 16.02). Determinations made on females of the stock colony from time to time have shown not the slightest indication of anemia among the colony.

In view of the fact that Waddell, Steenbock, Elvehjem and Hart (8) have recently reported a similar technique, a chart showing the growth curves and hemoglobin picture of several characteristic individuals on an exclusive whole milk diet is presented. It will be seen that this agrees entirely with the findings of the above-mentioned workers.

As indicated on the chart, the size of the animal when placed on the exclusive milk diet influenced the subsequent degree of growth and the rate at which the hemoglobin decreased. This has been found to be true generally. It is believed that when dealing with closely inbred stock the food consumption is as important as heredity in determining size. Hence, in the case of weanling rats of the same age, the larger ones have a greater iron reserve than the others. To insure the development of severe anemia in a short time rats should be transferred to the whole milk diet as soon after they have reached the stage when they start to consume some of the mother's ration as their weight permits.

SUMMARY

A procedure is described whereby studies in nutritional anemia may be made by using weanling rats from normal females. The method involves the use of an exclusive whole milk diet which brings about severe anemia in from four to six weeks. By supplementing the milk after the development of anemia, or from the beginning, and making regular hemoglobin determinations, the preventive or curative power of the substance under test can readily be determined. The results obtained agree closely with those recently reported by Waddell, et al. (8).

---

[1] Yellow cornmeal 67, oilmeal 12, casein 16, alfalfa meal 3, salt 1, calcium carbonate 1, milk *ad libitum*.

REFERENCES

(1) MATTILL, H. A., AND CONKLIN, R. E. 1920 The nutritive properties of milk, with special reference to reproduction in the albino rat. Jour. Biol. Chem., xliv, 137–158.

(2) MATTILL, H. A., AND STONE, N. C. 1923 The nutritive properties of milk, with special reference to reproduction in the albino rat. II. Jour. Biol. Chem., lv, 443–455.

(3) MATTILL, H. A., CARMAN, J. S , AND CLAYTON, M. M. 1924 The nutritive properties of milk. III. The effectiveness of the x substance in preventing sterility in rats on milk rations high in fat. Jour. Biol. Chem., lxi, 729–740.

(4) DANIELS, A. L., AND HUTTON, M. K. 1925 Mineral deficiencies of milk as shown by growth and fertility of white rats. Jour. Biol. Chem., lxiii, 143–156.

(5) MITCHELL, H. S., AND SCHMIDT, L. 1926 The relation of iron from various sources to nutritional anemia. Jour. Biol. Chem., lxx, 471–486.

(6) SCOTT, J. M. D. 1923 Studies in anaemia. II. Biochem. Jour., xvii, 166–169.

(7) BROUWER, E. 1926 Note on anaemia, urobilinuria and intestinal haemorrhage in rabbits in consequence of exclusive nutrition with cow's and goat's milk. Biochem. Jour , xx, 105–107.

(8) WADDELL, J., STEENBOCK, H., ELVEHJEM, C. A., AND HART, E. B. 1928 Iron in nutrition. V. The availability of the rat for studies in anemia. Jour. Biol. Chem., lxxvii, 769–775.

THE RELATIONS OF TEMPERATURE AND TIME OF FOREWARMING OF MILK TO THE HEAT STABILITY OF ITS EVAPORATED PRODUCT*

E. F. DEYSHER, B. H. WEBB AND GEORGE E. HOLM

Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture

It has been long known that heating a milk to boiling temperature prior to its evaporation markedly increases the heat stability of its evaporated product. Little is known, however, concerning the effect of temperatures other than those at or near the boiling point.

In a former publication (1) it has been shown that variations in the temperature of forewarming have little effect upon the heat stability of milks of normal solids concentration. Increases in the concentration of milk fat varied the stability but slightly except in cases wherein homogenization was used. In the latter cases various temperatures of forewarming were found to vary considerably the time of coagulation of the product. It had previously been noted that the variations in the stability of evaporated milks prepared from milks forewarmed to different temperatures were in some respects analogous to those obtained with normal milks of high fat content heated to similar temperatures and homogenized at various pressures.

EXPERIMENTAL

To determine the effect upon the stability to heat of increasing the solids-not-fat and fat contents of milk several milks of the following concentrations of solids were used:

(a) 9 per cent solids-not-fat
(b) 9 per cent solids-not-fat + 8 per cent milk fat
(c) 18 per cent solids-not-fat
(d) 18 per cent solids-not-fat + 8 per cent milk fat

---

* Received for publication August 1, 1928.

The time of coagulation of these samples at 120°C. was determined, and the averages of the results obtained are plotted in figure 1.

In view of the considerably greater susceptibility to coagulate of a milk of 18 per cent solids-not-fat content when sterilized at a temperature of 120°C. than of a milk of 9 per cent solids-not-fat concentration (1), the variations noted for the former product are significant. The results are also striking in that with the milks containing 18 per cent solids-not-fat there is a range of

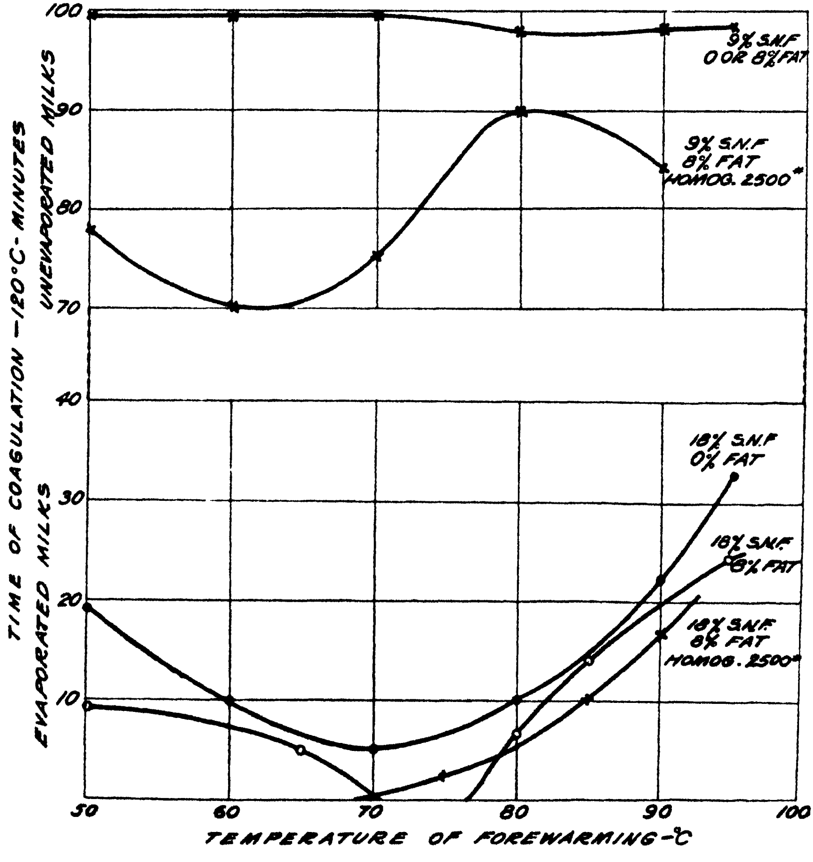


FIG. 1. THE RELATIONSHIP OF THE HEAT STABILITY OF MILKS OF DIFFERENT SOLIDS CONCENTRATIONS AND FOREWARMED AT DIFFERENT TEMPERATURES

variation of almost 100 per cent in the time of coagulation, when the forewarming temperature ranges from 70° to 95°C.

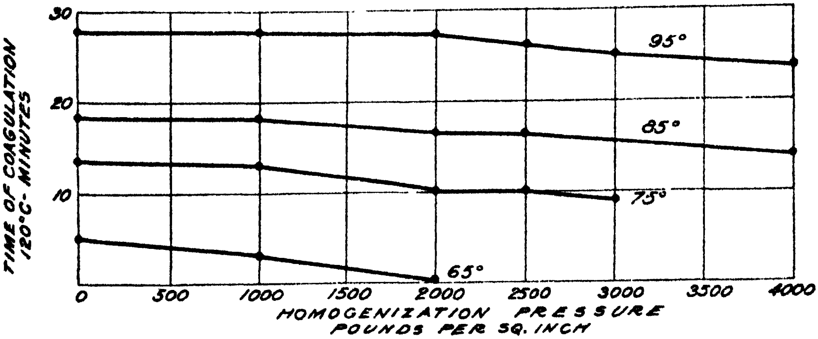


FIG. 2. EFFECT OF VARIOUS PRESSURES OF HOMOGENIZATION ON THE HEAT STABILITY OF EVAPORATED MILKS PREPARED FROM MILKS FOREWARMED AT DIFFERENT TEMPERATURES

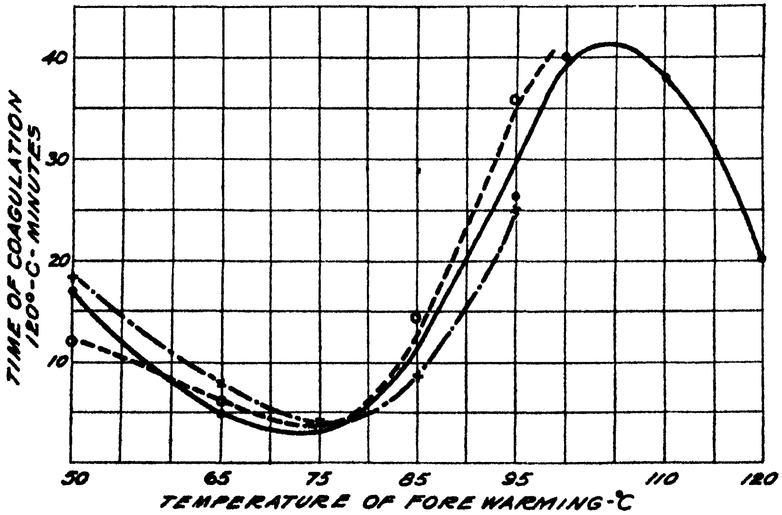


FIG. 3. THE EFFECT OF DIFFERENT FOREWARMING TEMPERATURES (TEN MINUTES) UPON THE HEAT STABILITY OF THREE EVAPORATED SKIM MILKS (18 PER CENT SOLIDS-NOT-FAT)

With creams, increased pressures of homogenization vary the time of coagulation to a marked degree (1). The lowered heat stability due to increased homogenization pressures, is also dependent upon the fat content, but becomes very slight for products containing less than 10 per cent milk fat. Experiments with several samples of an evaporated product containing 18 per cent solids-not-fat and 8 per cent milk fat show that increased homogenization pressure produces but slight effect upon the heat coagulation of the milk. Each sample remained nearly constant, in this respect, at a level determined by the forewarming treatment of the milk used. Figure 2 graphically represents the data obtained.

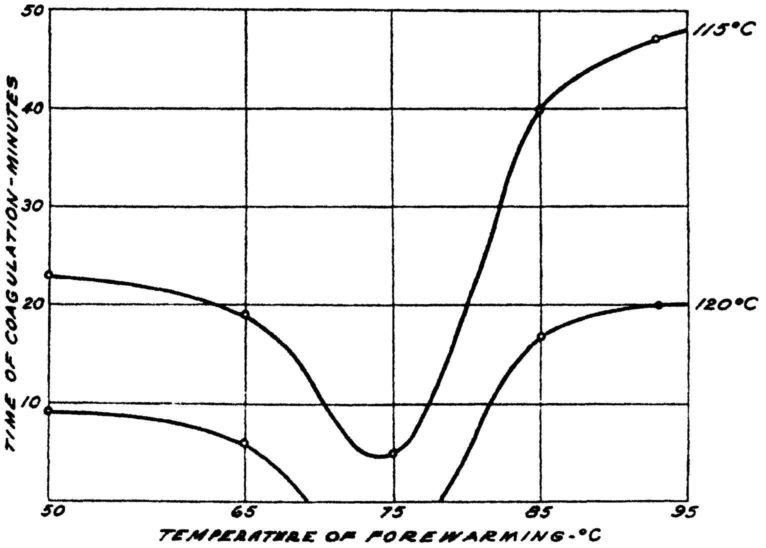


FIG. 4. THE EFFECT OF DIFFERENT TEMPERATURES OF FOREWARMING UPON THE HEAT STABILITY OF EVAPORATED MILK (18 PER CENT SOLIDS-NOT-FAT AND 8 PER CENT FAT)

The variations in the time of coagulation of evaporated products from milks forewarmed at different temperatures, indicated in figure 1, were sufficiently great to warrant closer study. Samples of a number of skim milks were forewarmed at 65°, 75°, 85°, and 95°C. for ten minutes, evaporated to 18 per cent solids-not-fat content, and the time of coagulation at 120°C. determined. Additional samples of one of the milks were forewarmed at 100°, and at 110°, and 120°C. under pressure for ten minutes and treated similarly. The results of some of these experiments are plotted in figure 3.

Although the presence of 8 per cent of milk fat, distributed in a form normal to milk, has little effect upon the stability to heat, it varies the magnitude of the changes noted for different forewarming temperatures in the samples containing no milk fat. The general nature of the variations is, however, the same.

Typical heat stability curves for evaporated whole milk are shown in figure 4.

TABLE 1

The effect of variations in the temperature of forewarming upon the time of coagulation of the evaporated product

SAMPLE NUMBER	TEMPERATURE OF FOREWARMING	TIME OF FOREWARMING	TIME OF COAGULATION AT 120°C.
	°C.	*minutes*	*minutes*
1	95	10	40
	110	10	43
	120	10	41
2	95	10	25
	100	10	40
	110	10	37+
3	95	10	40
	105	10	58

The results given indicate that temperatures of forewarming greater than those ordinarily used in the industry are beneficial in further stabilizing the resulting evaporated product.

In table 1 are given the results obtained with several samples of herd milk. These results also indicate that increases in the temperature of forewarming increase the heat stability of the evaporated product. It should be stated, however, that this is true only when the milk used is of excellent quality. With poorer grades of milk the heat applied in forewarming is additive to that of sterilization and hence shortens the time of coagulation.

The magnitude of increased stability with increases in forewarming temperatures, (applied for ten minutes) has been shown in figure 3. An increase in the heat treatment during forewarming may also be accomplished by a variation in the time factor. In table 2 is shown the time of coagulation of samples of an evaporated milk heated to 95°C. for varying periods of time.

With these particular milk samples long periods of forewarming at 95°C. were beneficial to the stability of the evaporated product to heat. Samples of milk of poorer quality, when forewarmed for periods longer than fifteen minutes, decreased in their heat stability.

Though an increase in the degree of heating does to a certain extent increase the stability of the evaporated product, the effect of this added treatment upon its body must be taken into account. Forewarming milk at high temperatures for extended periods of time decreases the body of the product and is, therefore, not practicable commercially.

TABLE 2

The effect of variations in the time of forewarming upon the time of coagulation of the evaporated product

SAMPLE NUMBER	TEMPERATURE OF FOREWARMING	TIME OF FOREWARMING	TIME OF COAGULATION AT 119°C
	°C.	*minutes*	*minutes*
1	95	10	27
	95	15	29
	95	20	31
	95	25	33
2	95	10	40
	95	20	52
	95	30	49

The data given in figure 3 indicate a gradual increase in stability with increases in temperatures of forewarming above 75°C. In a majority of the samples used, however, an interesting phenomenon was encountered; namely, heating at 90°C. for ten minutes prior to their evaporation produced evaporated products of greater stability than did heating at 95°C. for the same interval of time. Temperatures greater than 95°C. were as a rule found to be more beneficial to stability than was 95°C. A few of these results are given in figure 5.

The results given in this figure indicate also that only the milks of good quality are improved in heat stability when forewarmed at the higher temperatures.

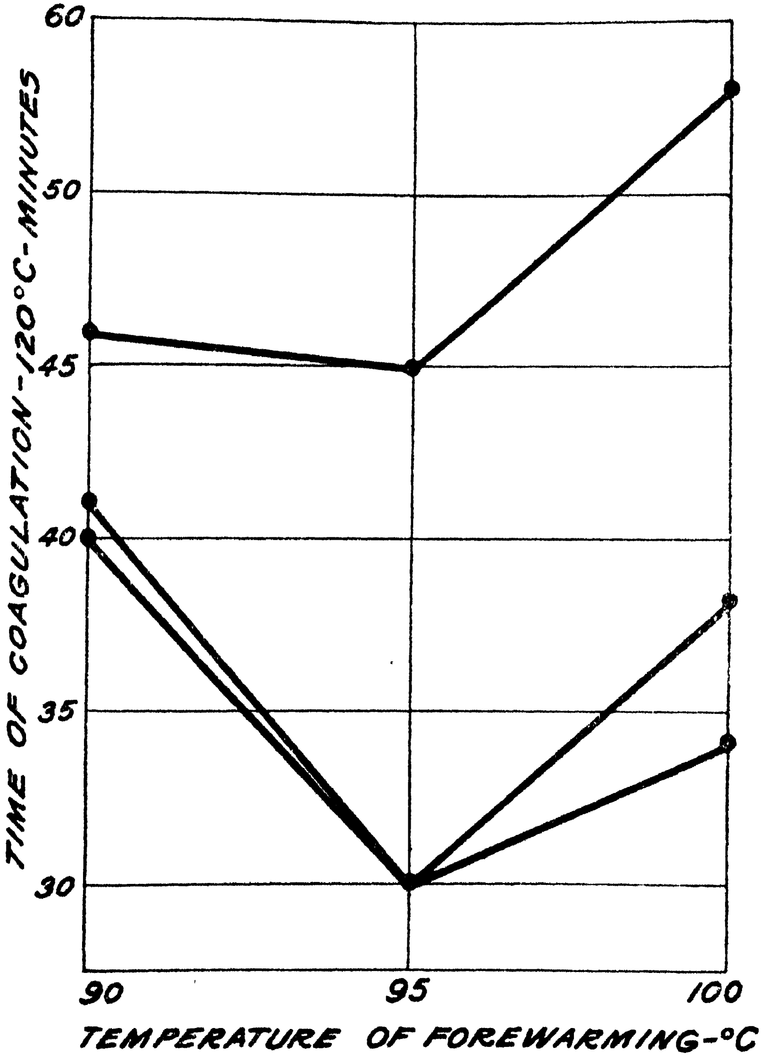


FIG. 5. THE EFFECT OF FOREWARMING AT 90°, 95°, AND 100°C. (TEN MINUTES) UPON THE HEAT STABILITY OF THE EVAPORATED MILKS

BODY OF EVAPORATED MILKS

Although stability to heat is the prime factor to be considered in the manufacture of evaporated milks, it is necessary that the body of the finished product be of a creamy consistency.

The thickening during sterilization is a part of the coagulation process and is of relatively short duration. All milks do not, however, attain the same body before visible curd formation begins. The body attained depends largely upon the quality of the milk although it may vary considerably with variations in the forewarming treatment. Preliminary experiments indicate that the relationship between the consistency attainable before coagulation begins and the stability to heat are of a reciprocal nature. In other words, although the milks forewarmed to 65° to 70°C. produced evaporated products of low stability they produced evaporated products of the greatest relative consistency. Those milks which produced evaporated products of greatest stability when forewarmed to 105°C. for ten minutes also produced products of the lowest relative consistency at the time of coagulation. It is obvious, therefore, that milks of exceedingly great stability to heat are not the most desirable from the standpoint of production of body.

The data obtained during this work are in accordance with the results obtained by Grinrod (3), who subjected the milk to the "impact process" of sterilization and found that milk heated to 110°C. for one minute possessed greater heat stability than did milk forewarmed at the ordinarily lower temperatures used commercially. The resulting evaporated milk possessed, however, a very fluid and undesirable body.

DISCUSSION

The data presented indicate that the heat stability of an evaporated milk is dependent upon several variable factors, the most important of which is the temperature of forewarming. The process of homogenization affects stability to a lesser degree. Variations in the solids-not-fat content or in the milk fat content have marked effects upon the magnitude of the changes brought about by forewarming and homogenization. Homogenization of a milk containing 8 per cent fat produces a distinct change in the heat stability at the various forewarming temperatures. The effect of increasing the solids-not-fat content, while it lowers the stability, does not markedly change the form of the characteristic stability curve obtained when homogenization is used.

The chief difference noted between the heat stability curves of an unevaporated homogenized milk of 8 per cent fat content and of an evaporated milk, is that their points of minimum stability occur at different temperatures of forewarming. (See figure 1.)

Homogenization causes lowest stability at a forewarming temperature of 60° to 65°C. Increases of solids-not-fat to 18 per cent produce a minimum stability of unhomogenized milks when a forewarming temperature of 70° to 75°C. is used.

Ionic equilibria are intimately connected with the stability of colloid solutions. Sommer and Hart (4) believe that the heat stability of milk is dependent upon the ratio of the amount of calcium and magnesium to that of the phosphates and citrates, or the "salt balance." Though this may hold true for milks which have not been forewarmed, concentrated or homogenized, the effect of the redistribution of phases must be considered in evaporated products. A normal milk of 8 per cent fat content shows slight variations in heat stability resulting from different forewarming temperatures (figure 1), while the same milk when homogenized shows the same general variations in heat stability as does its evaporated unhomogenized product.

No explanation is offered for the peculiar effect of homogenization on the heat stability of a milk of high fat content forewarmed at different temperatures. As already suggested in the case of homogenized cream there may be a variation in potential upon the fat globules, resulting in increased susceptibility to coagulation. In order to attempt an explanation of the effect of concentration upon heat stability more data upon the equilibria involved are necessary than are now available.

SUMMARY

Variations in the heat treatment of a milk affect greatly the heat stability of the evaporated product. Temperatures up to 70°C., applied for ten minutes decrease the stability, while higher temperatures markedly increase it. Higher temperatures and a longer heating period than those generally used further stabilizes the product to heat. This treatment is objectionable, however, in that the body of the resulting product is affected adversely.

Homogenization of an evaporated milk containing 18 per cent solids-not-fat and 8 per cent fat at pressures up to 4000 pounds per square inch affects its heat stability but slightly.

REFERENCES

(1) WEBB, B. H , AND HOLM, G E. 1928 The heat stability and feathering of sweet cream, as affected by different homogenization pressures and different temperatures of forewarming Jour. Dairy Sci., xi, 243.

(2) HOLM, G. E., DEYSHER, E. F., AND EVANS, F. R. 1923 The relationships of concentration and time to the temperature of coagulation of evaporated skim and whole milk. Jour. Dairy Sci., vi, 556.

(3) GRINROD, G. E. 1927 Sterilization of milk by impact of steam. Creamery and Milk Plant Monthly, xvi, no. 9.

(4) SOMMER, H. H., AND HART, E. B. 1928 Heat coagulation of evaporated milk Research Bull. 67, Agr. Exp. Sta., University of Wisconsin.

THE NUTRITIONAL VALUE OF COPPER IN POWDERED WHOLE MILK*

R. W. TITUS AND J. S. HUGHES

Department of Chemistry, Kansas State Agricultural College, Manhattan, Kansas

Much has been written on the deleterious effect of copper on the nutritional value, flavor, and keeping quality of condensed and powdered milk. Manufacturers have even taken steps to substitute other material for copper in the construction of vacuum pans.

Researches of Supplee and Bellis (1), Quam and Hellwig (2) and others have demonstrated the presence of small amounts of copper in normal milk while Hart and co-workers (3) have shown very conclusively that the quantity of copper in normal milk when fed with pure iron, is too small for normal growth and hemoglobin production. However, if small amounts of copper are added to a milk-iron diet, rats respond by increased growth and hemoglobin generation.

Rice and Miscall (4) and Rice (5) have shown that copper is dissolved from the vacuum pan during the condensing process in the manufacture of condensed and powdered milk, thus increasing the quantity of copper in these products to an abnormal amount. Hess and Unger (6) suggest that traces of copper may act as a catalyzer causing destruction of the antiscorbutic vitamin in the product.

Experiments in our laboratory have shown that rats grow and form hemoglobin normally when fed iron plus whole milk powder which has had its copper content increased by condensation in a copper vacuum pan. Rats given whole milk powder without the iron fail to grow and rapidly become anemic.

EXPERIMENTAL

The young rats used in this work were started on experiment at weaning time when twenty-five to twenty-seven days of age.

---

* Received for publication August 20, 1928. Contribution No. 143. Department of Chemistry.

The mothers had been fed a stock ration consisting of whole wheat and milk powder.

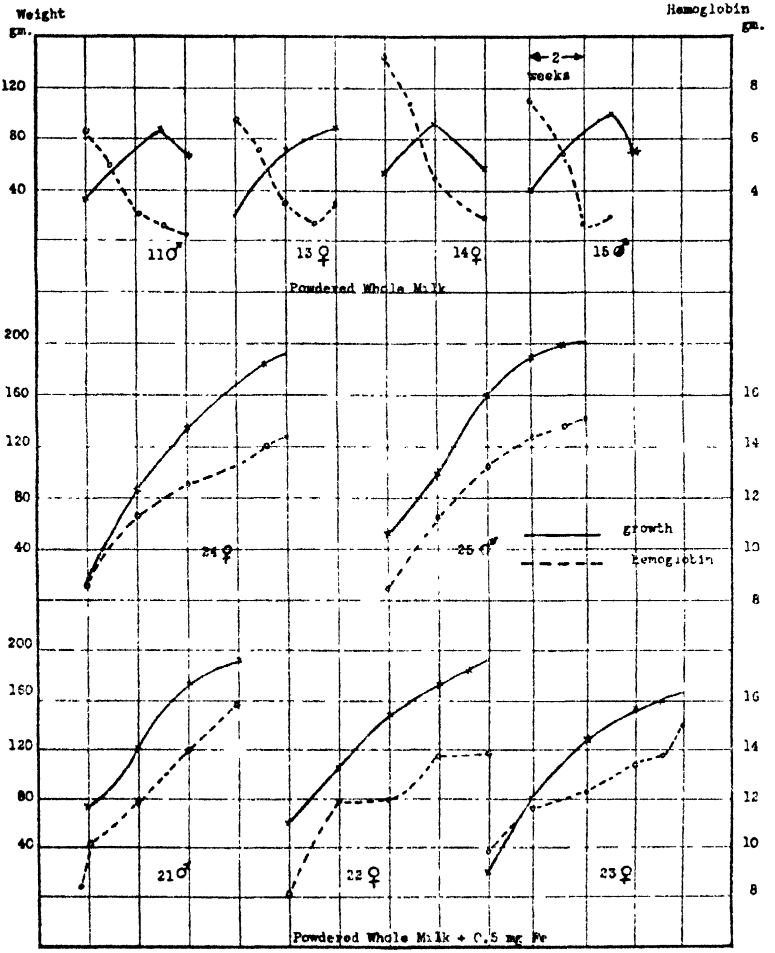


FIG. 1. SHOWING GROWTH AND HEMOGLOBIN CURVES FOR RATS ON WHOLE MILK POWDER AND WHOLE MILK PLUS IRON

The whole milk powder contained increased amounts of copper due to the solvent action of the milk on the copper vacuum pan.

The animals were ear-marked and placed in individual cages on screens, distilled water was used in mixing the dried milk and porcelain mortars were used for the food receptacles. Each animal was weighed and hemoglobin determinations made weekly. One drop of cod liver oil per day was given each rat in order to insure a sufficient amount of vitamins A and D in the ration.

Figure 1 shows growth curves and grams of hemoglobin per 100 cc. of blood on four animals receiving powdered whole milk and on five animals receiving powdered whole milk plus iron. The animals receiving only the powdered whole milk failed to grow and rapidly became anemic while the animals receiving the whole milk powder plus the iron showed good growth and hemoglobin production throughout the experimental period of ten weeks.

The iron used was a special Mallinckrodt iron wire for "standardization." The iron was dissolved in HCl, then treated with H_2S, under pressure for about twelve hours, in order to remove any trace of copper which might be present. The powdered milk contained about 0.0006 per cent copper as determined by the Biazzo method (7). Each rat when two months of age consumed about 8 grams of the milk powder, giving it an equivalent of approximately 0.05 mgm. of copper daily. Hart (3) found the optimum amount of copper per rat per day to be about 0.05 mgm.

CONCLUSIONS

1. Increased copper content of whole milk powder, due to the solvent action of the milk on the copper vacuum pan, instead of being altogether detrimental, may be beneficial.
2. Rats, fed a whole milk powder plus 0.5 mgm. iron showed excellent growth and hemoglobin production throughout an experimental period of ten weeks.
3. This experimental work suggests a possible value of powdered whole milk in infant feeding, however, no experimental work with copper on human subjects has as yet been reported.

REFERENCES

(1) Supplee, G. C., and Bellis, B.: The copper content of cow's milk. Jour. Dairy Sc., 1922, v, 455.

(2) Quam, G. N., and Hellwig, Arthur: The copper content of milk. Jour. Biol. Chem., 1928, lxxviii, 681.

(3) Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A.: Copper as a supplement to iron for hemoglobin building in the rat. Jour. Biol. Chem., 1928, lxxvii, 797.
(4) Rice, F. E., and Miscall, J.: Copper in dairy products and its solution in milk under various conditions. Jour. Dairy Sc., 1923, vi, 261.
(5) Rice, F. E.: Sweetened Condensed Milk. VI. Tallowiness. Jour. Dairy Sc., 1926, ix, 459.
(6) Hess, A. F., and Unger, L.: Proc. Soc. Exptl. Biol. Med., 1921, xix, 119.
(7) Biazzo, R.: Ann. Chim. applicata, 1926, xvi, 96.

THE APPLICATION OF STEAM FOR HEATING AND STERILIZING DAIRY EQUIPMENT*

A. W. FARRALL

Department of Agricultural Engineering, University of California, Davis

Steam is quite commonly used for heating and sterilizing dairy equipment, both on the farm and in the dairy manufacturing plant. It is used in the small self contained oil or electric sterilizers and in large continuous can washers. Bacteriologists tell us that a certain temperature maintained for a certain length of time is effective in killing bacteria. It is the problem then of the engineer to determine the best methods of applying steam to the utensils in order to heat them most thoroughly, quickly and economically. A study of this problem has been made at the University of California, by the author in coöperation between the Divisions of Dairy Industry and Agricultural Engineering. Many tests have been conducted with the small dairy farm sterilizer, with large continuous can washers in plants and with a special testing apparatus for determining the rate of heating of 10-gallon milk cans over steam jets. Most of the data presented in this discussion were obtained with the last named apparatus. It may also be stated that this is in the nature of a progress report only, since there are several phases of the problem which have not been attacked as yet. It is believed however that since the data in this paper are more or less fundamental to the whole subject, it should be called to the attention of the industry at this time.

In outlining the problem, one might consider:

First, the thermal properties of steam, and the fundamentals of heat transfer from steam to metal surfaces and from metal surfaces to air, which are involved in the study.

Second, practical applications, such as the steaming in tank

---

* Received for publication August 20, 1928. Presented at the meeting of The American Dairy Science Association, Madison, Wisconsin, June 27, 1928.

type sterilizers in which the utensil is surrounded on all sides both inside and out with the heating medium, or the case of the utensil being steamed over a jet in which the outside of the can may be in contact with air or steam depending upon conditions and whether it is steamed in a continuous washer or a simple can steamer.

Third, the matter of moisture left on the utensil after steaming, since any moisture left in the finished can may cause rusting of metal surfaces and growth of bacteria.

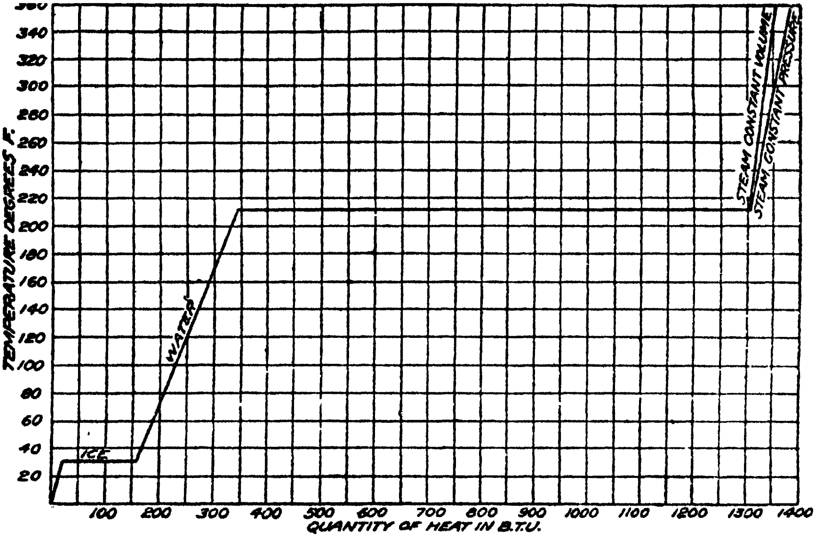


FIG. 1. HEAT-TEMPERATURE DIAGRAM FOR WATER

(From U. S. D. A. Bulletin No. 98)

THE PROPERTIES OF STEAM WHICH ARE OF IMPORTANCE IN THE STEAMING OF DAIRY UTENSILS

A study of the heating of dairy utensils would be incomplete without some discussion of the properties of the heating medium used, and therefore some of the more important thermal properties of steam are mentioned as follows:

The heat-temperature diagram (fig. 1) for water shows that considerable quantities of heat are absorbed as the water changes

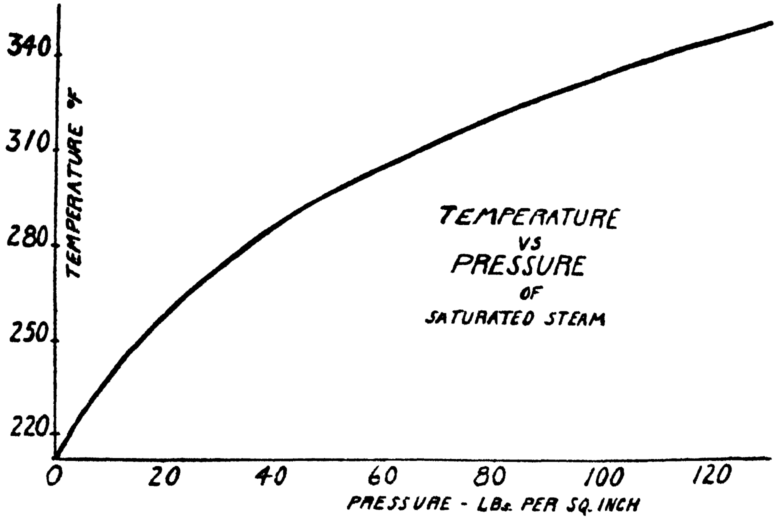


FIG. 2. CHART SHOWING HOW THE TEMPERATURE OF SATURATED STEAM VARIES WITH THE PRESSURE

(From Bureau of Standards Table)

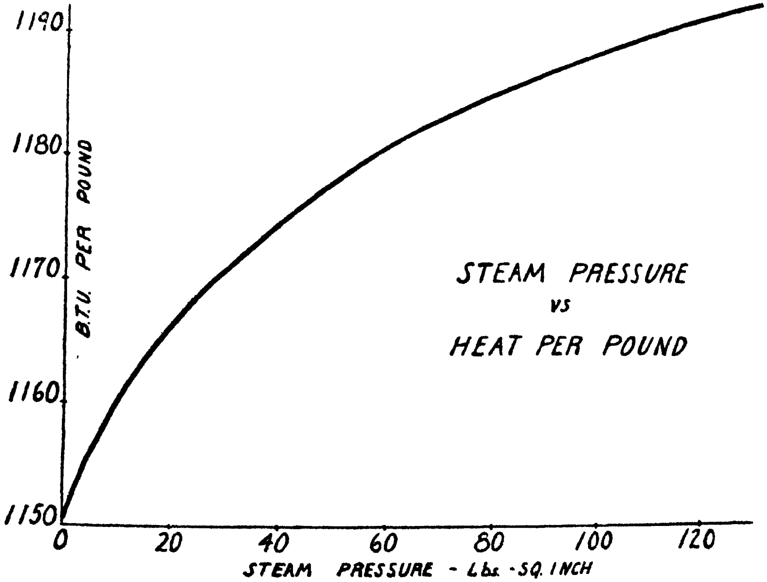


FIG. 3. CHART SHOWING HOW THE TOTAL HEAT IN A POUND OF SATURATED STEAM VARIES WITH THE PRESSURE

(From Bureau of Standard Table)

to steam, at the temperature of the boiling point. This heat is all given up again as the steam condenses and changes back to its original state. It should be mentioned that because this condensing temperature is high, and the quantity of heat liberated is great, steam is an especially desirable heating agent.

Another point worthy of note is that the temperature of saturated steam varies with the pressure. (See fig. 2.) This saturated

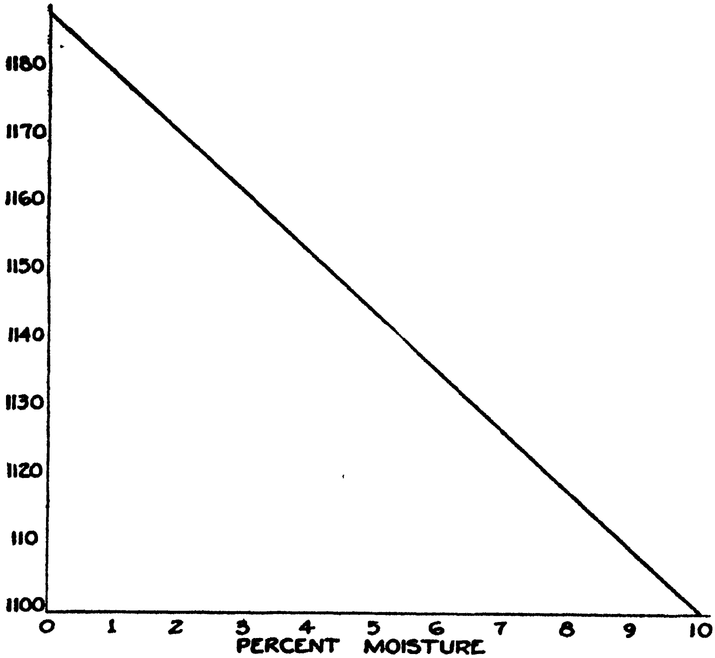


FIG. 4. CHART SHOWING THE RELATIONSHIP OF THE HEAT CARRYING CAPACITY OF STEAM WITH ITS MOISTURE CONTENT

steam at atmospheric pressure has a temperature of 212°F. approximately, while at 100 pounds pressure, the temperature is 337.9°F.

The heat content of saturated steam also varies with the pressure. The steam at high pressure carries slightly more heat per pound than it does at low pressure. (See fig. 3.)

The heat carrying capacity of steam is also dependent upon the quality, thus saturated steam which carries 1186 B.t.u. per pound at a certain pressure and temperature carries only 1100 B.t.u. when it contains 10 per cent moisture. (See fig. 4.) The same steam if superheated 90°F. would carry approximately 1228 B.t.u. per pound.

Superheated steam has the power of taking up moisture. Thus if steam is superheated 200°F. it will absorb approximately .1 pound of moisture, per pound of steam, the exact amount depending upon the pressure of the steam. (See fig. 5.)

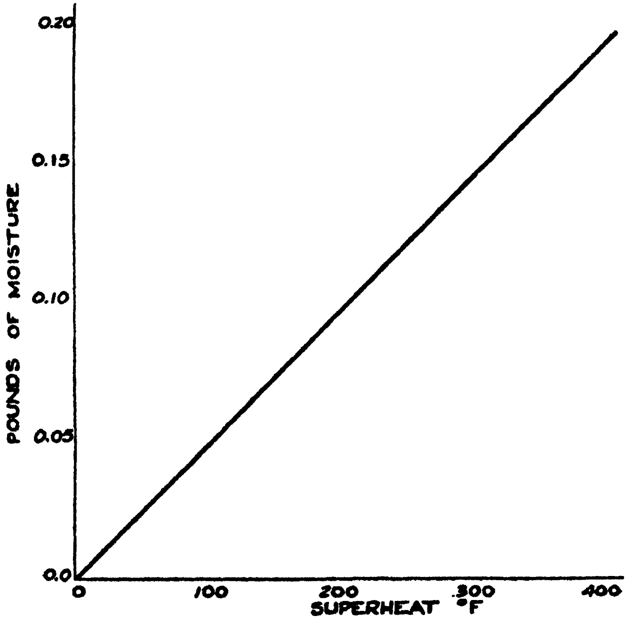


FIG. 5. CHART SHOWING THE MOISTURE ABSORBING POWER OF SUPERHEATED STEAM WITH VARIOUS DEGREES OF SUPERHEAT

THERMODYNAMICS INVOLVED IN STEAMING DAIRY UTENSILS

Heating utensils with steam or other media of like nature involves certain laws of heat transfer, since it is always a case of transferring heat from the medium to the utensil. The heat transferred to the utensil must be sufficient to raise the temperature of the utensil and in some cases supply a certain amount additional to care for that radiated into the air. Steaming inside a closed tank does not involve this loss, but involves radiation losses from the tank. An example of severe losses from radiation to the atmosphere is noted when a piece of equipment is heated by blowing steam against it from the outside.

It has seemed advisable to work out certain equations to serve as a guide in predicting results of steaming utensils. It should be borne in mind, however, that some of the constants used in the equations will vary for different temperatures, quality of steam, etc. The equations follow:

Equation I. Heat absorbed by a utensil during temperature rise.

$$H = (t_2 - t_1) \text{ sp. ht.} \times W \qquad \text{I}$$

where H = heat absorbed with a temperature rise $t_2 - t_1$, in B.t.u.
t_2 = final temperature, degrees Fahrenheit.
t_1 = original temperature, degrees Fahrenheit.
sp. ht. = sp. heat of utensil = B.t.u. per degrees Fahrenheit change per pound of utensil.
W = weight of utensil, pounds.

Equation II. To find pounds of steam required to heat utensils (considering 100 per cent efficiency).

$$\text{(IIA)} \quad S = \frac{W \times (t_2 - t_1) \times \text{sp. heat}}{(h + qL) - (t_c - 32)} \text{ using wet or saturated steam}$$

or

$$\text{(IIB)} \quad S = \frac{W \times (t_2 - t_1) \times \text{sp. heat}}{h + L + C_p\,(t_{\text{sup}} - t_{\text{sat}})} - (t_c - 32) \text{ using superheated steam}$$

where S = steam required, pounds.
W = weight of utensils, pounds.
t_2 = final temperature of utensil, degrees Fahrenheit.
t_1 = original temperature of utensil, degrees Fahrenheit.
Sp. ht. = sp. heat of utensil = B.t.u. per degree Fahrenheit change per pound utensil.

h = heat of liquid of steam, B.t.u.
Q = quality of steam. Saturated = 1.00.
L = latent heat of vaporization of steam at pressure, B.t.u. per pound.
t_c = temperature at which steam condensate is removed, degrees Fahrenheit.
t_{sup} = temperature of superheated steam, degrees Fahrenheit.
t_{sat} = temperature of saturated steam at pressure given, degrees Fahrenheit.

Equation III. Heat balance at equilibrium, utensil heated over a steam jet.

$$K_1 (t_3 - t_2) = K_2 (t_2 - t_4) \tag{III}$$

where K_1 = heat transfer coefficient for steam to metal of utensil, B.t.u. sp. ht./min./deg. diff. in temperature between steam and metal.
K_2 = heat transfer coefficient from external surface of metal to surrounding medium—air or vapor. Given in B.t.u./sq. ht./min./deg. diff. in temperature.
t_3 = temperature of steam, degrees Fahrenheit.
t_2 = temperature of can, degrees Fahrenheit.
t_4 = temperature of medium outside of can degrees Fahrenheit.

At equilibrium, the heat received from the steam $K_1 (t_3 - t_2)$ per unit area and time will equal the heat lost by radiation to the outside or $K_2 (t_2 - t_4)$.

Equation IV. To find time required to heat utensils submerged in steam heating medium.

$$T = \frac{(t_2 - t_1)\,(\text{sp. heat})\,W}{K_1 \left[\left(t_3 - \frac{(t_2 - t_1)}{2}\right)\right] \times S_1} \tag{IV}$$

where T = time in minutes.
S_1 = surface of utensil exposed to steam, square feet.

Equation V. To find time required to heat utensil which is being steamed over jet.

$$\text{(V)} \quad T = \frac{(t_2 - t_1)\ \text{sp. heat} \times W}{\left[S_1 \times K_1\left(t_3 - \frac{(t_2 - t_1)}{2}\right)\right] - \left[K_2\left(\frac{(t_2 - t_1)}{2} - t_4\right) \times S_2\right]}$$

where T = time, minutes.

S_1 = Surface of can exposed to steam, square feet.

S_2 = Surface of can exposed to air, sq. ft.

All other symbols are described above.

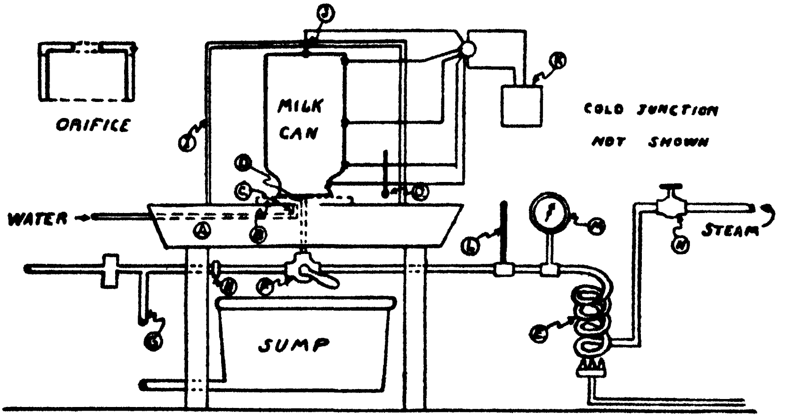


FIG. 6. DIAGRAM OF EXPERIMENTAL APPARATUS FOR STEAMING MILK CANS

TEST OF HEATING 10-GALLON MILK CANS OVER STEAM JETS

In order to study the effect of different factors on the heating of can when steamed over jets, a special apparatus shown in figures 6 and 7, was built. The essential features of this apparatus were as follows: A table, A, and an adjustable frame, B, to support the can and other parts, a water jet, C, which could be turned on and off so that the can could be easily cooled. A steam jet, D. made with a round orifice which could be easily changed for size, a superheating coil, E, heated by a gas flame for controlling the quality and temperature of the steam; a 3-way valve, F, which could be used for turning the steam quickly from the main steam jet, D, to a similar jet, H, in order to have a practically continuous and steady flow of steam through the superheater, *E*, so that its temperature and quality characteristics might be better controlled. The waste jet, *H*, carried off the waste steam. The housing, *I*, surrounded the can. Thermocouples, *J*, were soldered onto the can and connected to the millivoltmeter, *K*, and to a cold junction, kept at 32°F. Steam gauge, *M*, and thermometers, *L*, and, *O*, completed the arrangement.

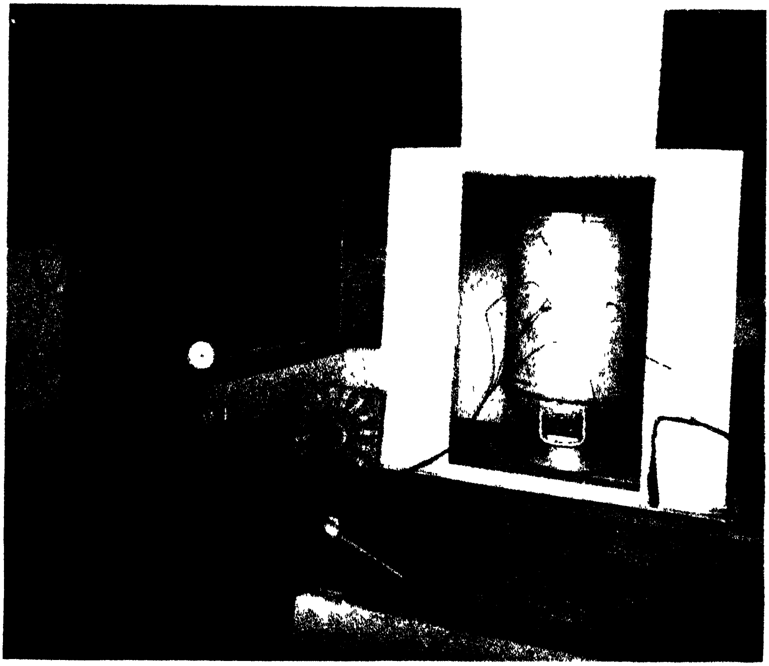


FIG. 7 PHOTOGRAPH OF APPARATUS USED FOR STEAMING MILK CANS

With this apparatus the steaming of the cans was quite closely controlled.

The general method of procedure during the tests was to place the can in position above the jet, turn on the steam to warm the can, then turn off the steam and turn on the cold water to bring the can temperature down to normal. The cycle was followed several times until the readings became constant, after which the official test was made.

Readings of the millivoltmeter were taken at five- and ten-second intervals for the period of one minute. There was some lag in the movement of the needle but it was very small, as the heat capacity of the thermocouples was small. Some practice was required in order to take the reading accurately, but this was accomplished and different tests checked closely. All data were checked at least three times. The thermocouples were moved so that different positions on the can were checked.

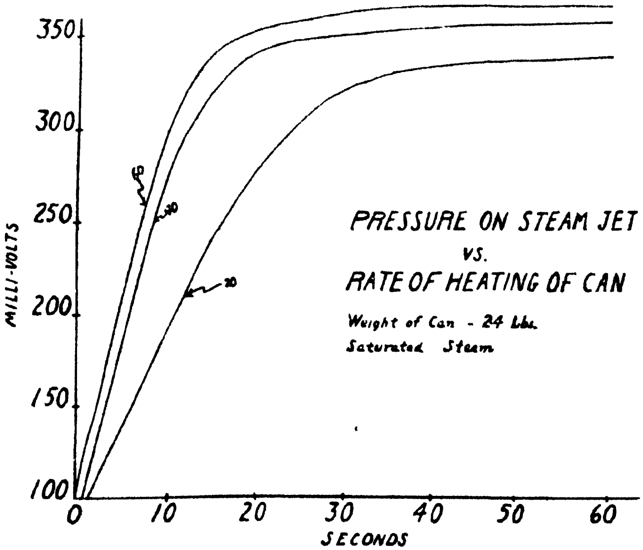


Fig. 8. Chart Showing the Effect of Various Pressures of Steam Jet upon the Rate of Heating of 10-Gallon Milk Can (350 M.V = 183°F.)

EFFECT OF STEAM PRESSURE ON RATE OF HEATING

Figure 8 shows typical curves obtained when the pressure was 20, 40, and 60 pounds respectively on the 0.25 inch nozzle, and when saturated steam was used. It is significant that the rate of heating was markedly slow when only 20 pounds pressure was used, whereas there was not much difference in the rate between 40 and 60 pounds pressure. This shows that with the 20 pounds pressure, steam was not admitted fast enough to maintain the saturation temperature of the steam within the can, during the first forty seconds, whereas with both the 40 and 60 pound pressures, the temperature was maintained practically after the first twenty seconds. It shows that the temperature in all cases finally reached a point of equilibrium at which it remained.

The practical significance of these data is that they emphasize the necessity for a large momentary volume of steam at the jet to bring the can to the proper temperature quickly, and that this volume could be decreased markedly in the interests of economy after attaining the proper temperature. A valve operated by a can in such a way that it opened quickly at first and gradually closed would be desirable. It is only a waste of steam to allow a heavy flow to continue after the can has been brought to the proper temperature.

EFFECT OF QUALITY OF STEAM UPON RATE OF HEATING

Figure 9 shows data obtained when the quality of steam was varied from wet to dry superheated, keeping all other factors constant. Steam pressure was 40 pounds and the orifice was 0.25 inch. It is interesting to note that the rate of warming of the can was very nearly the same for all during the first fifteen seconds although the very high temperature superheated steam showed somewhat more rapid heating during the first thirteen seconds. From these data it is clearly shown that wet or saturated steam heats the can more readily per degree temperature difference between it and the can than does superheated steam, however the latter will give higher final temperature if it is left on long enough. The conductivity of heat from wet steam to metal is better than from superheated steam to metal.

EFFECT OF QUALITY OF STEAM UPON AMOUNT OF MOISTURE LEFT IN CANS AFTER STEAMING

According to theory, superheated steam should have the power of absorbing moisture from the surface of the can or at least preventing an excessive amount of moisture from remaining in the can after steaming.

Figure 10 shows the results of tests, conducted in duplicate to show moisture remaining in the can after steamng for sixty

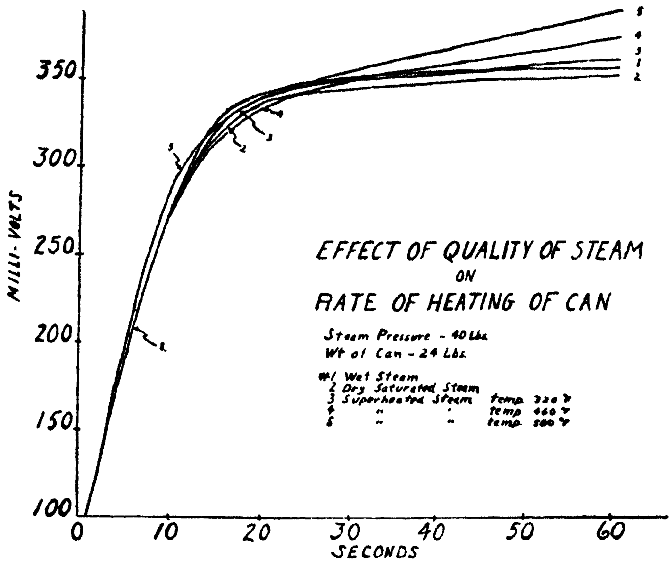


FIG. 9. CHART SHOWING HOW THE RATE OF HEATING OF A 10-GALLON MILK CAN VARIED WHEN STEAM OF DIFFERENT QUALITY WAS USED IN THE STEAM JET

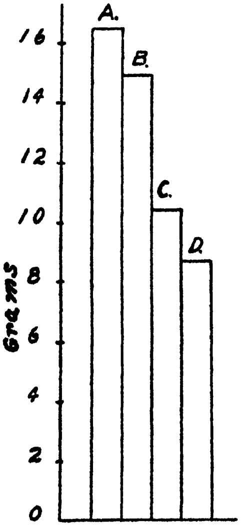


FIG. 10. CHART SHOWING GRAMS OF WATER LEFT IN 10-GALLON MILK CANS AFTER STEAMING OVER A $\frac{1}{4}$ INCH STEAM JET FOR SIXTY SECONDS

Note that the superheated steam left much less than did wet or saturated steam.

A. Wet steam at 278°F. B. Dry steam at 296°F.

C. Superheated to 420°F. D. Superheated to 532°F.

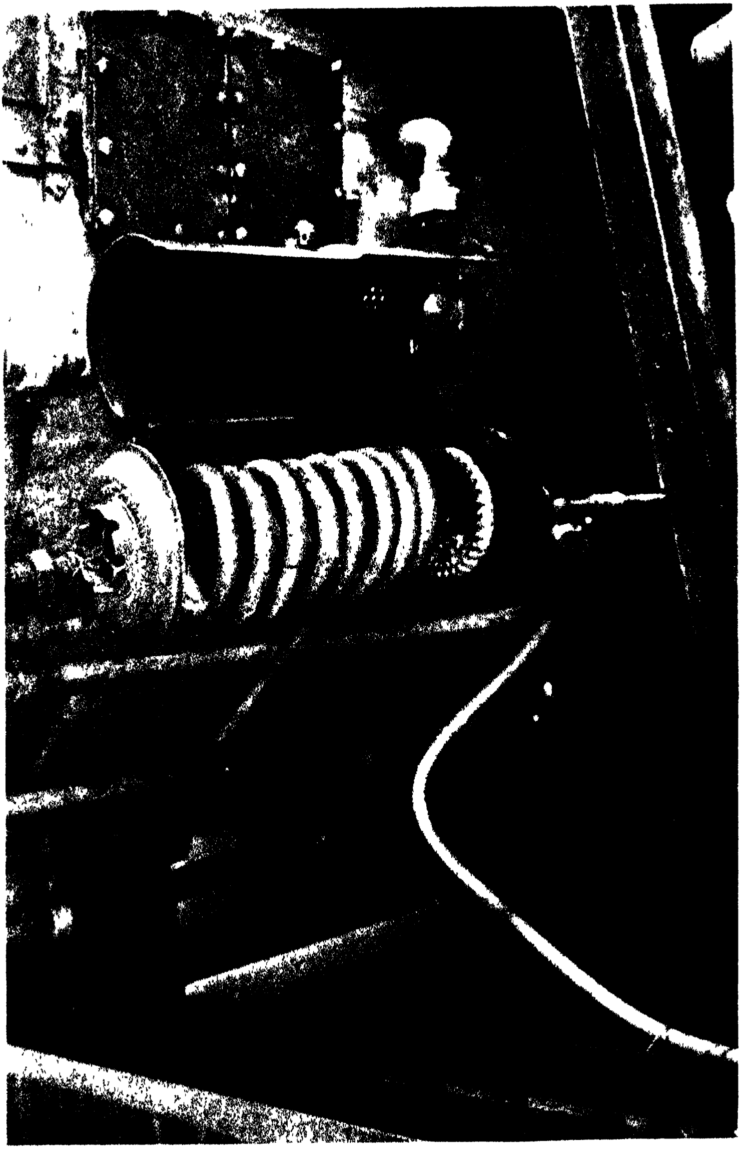


FIG. 11 PHOTOGRAPH OF A GAS HEATED SUPERHEATER ATTACHED TO THE FINAL STEAM JET OF A ROTARY CONTINUOUS CAN WASHER

seconds, using the various qualities of steam indicated. It is noted that with the wet and dry saturated steam the water was 16.30 and 14.45 grams, respectively, while with superheated steam this was cut down to 10.20 and 8.47 grams per can depending upon the temperature. The importance of this point is in the fact that if superheated steam is used in the last steam jet of a

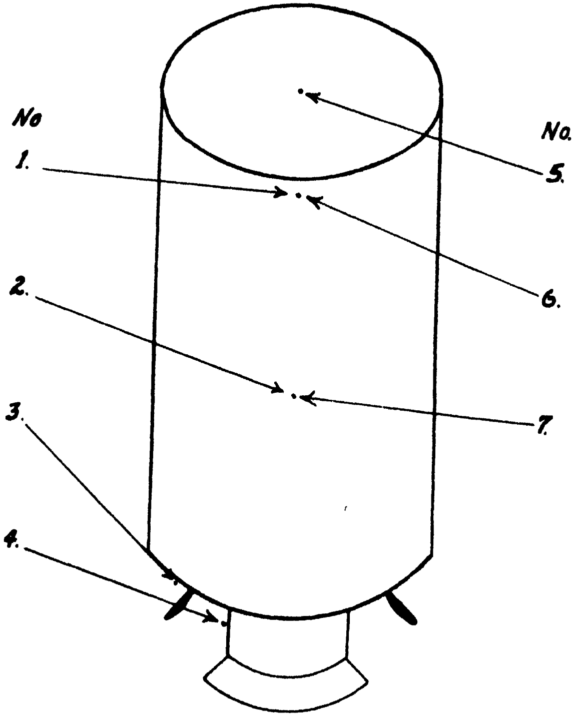


FIG. 12. CHART SHOWING POINTS AT WHICH THERMOCOUPLES WERE PLACED FOR MEASUREMENT OF VARIATION IN CAN METAL TEMPERATURE

can washer the drying process is materially aided since less moisture is left to be removed by the air drying apparatus.

This point was further tested by attaching a gas heated superheater (fig. 11) to a can washer and noting its effect. When using the superheater which furnished superheated steam for the last steam jet in a regular continuous commercial can washer, the cans usually came out with a parched dry appearance and very seldom with any moisture present. Considerable improvement was noted in the quality of drying small St. John's cans when the superheater was used. On the washer studied the regular air dryer nozzle was so large that it practically covered the mouth of the can and prevented free circulation of air up into it. However, the small high pressure jet of superheated steam easily entered the can, and carried out most of the water. It seems that more positive drying could be brought about if the drying air was forced in under relatively high pressure and forced through a smaller jet to give it sufficient velocity to travel to the top of the can and more thoroughly "scrub" the metal.

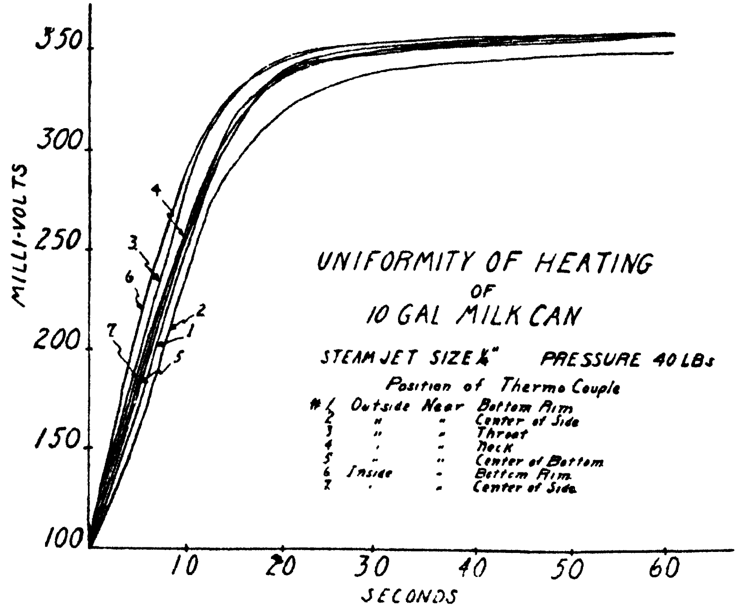


FIG. 13. CHART SHOWING THE VARIATION IN THE TEMPERATURE OF THE METAL OF A 10-GALLON MILK CAN DURING THE PROCESS OF HEATING OVER A JET OF SATURATED STEAM

UNIFORMITY OF HEATING OF THE CAN OVER STEAM JETS

With the same apparatus, tests were conducted to measure the uniformity of heating of the can metal, and points were established as shown in figure 12 for the location of the thermocouples as listed in figure 13. All were soldered to the outside of the can metal except nos. 6 and 7 which were carried through a small hole and soldered to the inside of the can at the same time closing the hole. Knowing the relatively high conductivity of the can metal, it would appear that the difference in rate of heating would not be appreciable with the type of jet used, since the steam was distributed quite thoroughly and there was no one place which received a strong current of steam as if a concentrating type jet were used. Figure 13 shows the results obtained. It is evident that the difference in the rate of heating is not great but that the most rapidly heating part is in the corner near the junction of the can bottom and side. This seems natural since it is where the condensate which is practically as hot as the steam, will tend to concentrate during the steaming process. Position 7 heated most slowly evidently due to the fact that radiation from this part of the can is quite marked, and also, there is a point where

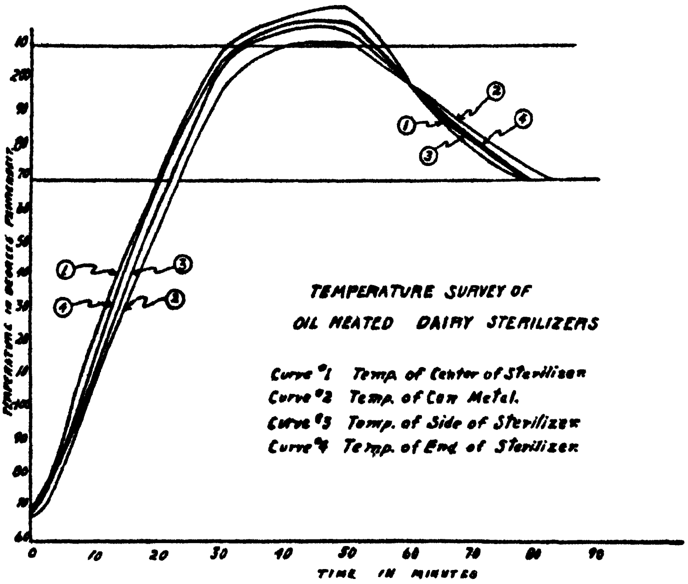


Fig. 14. Chart Showing the Temperatures of the Steam and the Can Metal When Using a Tank Type Oil Heated Sterilizer in Which Steam Was the Connecting Medium

cold air may strike, if it is drawn in with the steam. The temperature of the can metal varied 14.6°F. at the end of a fifteen-second steaming period, but only 5.7°F. after a thirty-second period. Further work is needed to measure these temperatures when other type of jets are used.

HEATING OF UTENSILS IN A CLOSED BOX

In certain types of sterilizers the utensil is heated by being placed in a chamber filled with steam. In this case the heat is applied to both inside and outside; turbulence is often poor, yet quite rapid heating takes place. It is usually not necessary to heat so quickly when this method is used. Figure 14 shows time-temperature curves which indicate the rate of increase of the temperature of the can and of the sterilizing chamber. It is evident that the rate of heating of the can follows quite closely the rate of heating of the sterilizer. Figure 15 shows the results of a similar test when using hot air instead of steam as the heating medium. It will be noted that there is a considerable lag in the temperature of the can as compared with that of the heating medium in the sterilizer.

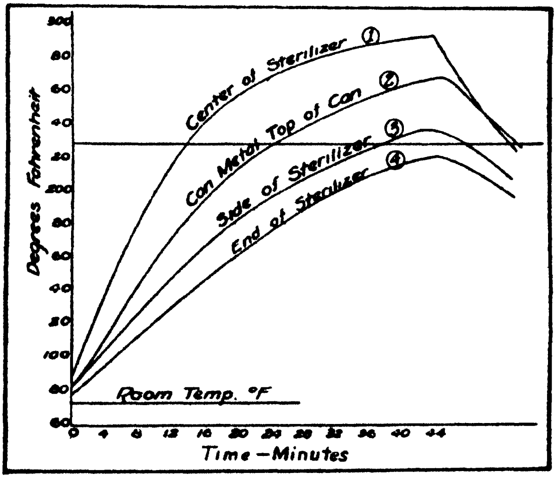


FIG. 15. CHART SHOWING THE TEMPERATURES OF THE HOT AIR AND THE CAN METAL WHEN USING AN ELECTRICALLY HEATED TANK TYPE STERILIZER IN HOT AIR WHICH WAS THE CONNECTING MEDIUM

SUMMARY

1. Commercial applications of steam for sterilizing might be classified as first, steaming of cans over jets, second, steaming of cans in tank type containers, third, steaming by blowing a steam jet against the outside of the metal surface.

2. The most important properties of steam which affect its use as a sterilizing medium are as follows:

a. Temperature of saturated steam increases with pressure.

b. Temperature of steam at any given pressure may be raised by superheating.

c. Superheated steam absorbs moisture.

3. Equations are developed which state that relationship between the factors involved in the thermal exchange which takes place when milk cans are steamed.

4. The heat absorbing capacity of a milk can is limited by the area of the surface, the coefficient of heat transfer, and the temperature difference between it and the heating medium. It is unnecessary to use any more steam than just enough to maintain the temperature of the heating medium inside the can, and to give the necessary turbulence, to insure a good coefficient of heat transfer.

5. Wet and saturated steam heated the cans at higher rates per degree difference in temperature between the can and the steam than did the superheated steam. Higher final temperatures of the can were obtained when superheated steam was used.

6. With a spreading type jet made by drilling a hole in a $\frac{1}{2}$ inch pipe capacity, the temperature in the can metal varied about 14.6°F. after 15 seconds steaming, and varied about 5.7°F. at the end of a half minute steaming period. The hottest part of the can was near the junction of the sides and bottom.

7. Steaming with superheated steam left much less moisture in the can than when saturated or wet steam was used.

8. The use of superheated steam in the last steam jet of a continuous can washer assists in drying the cans.

9. The time-temperature curve showing the rate of temperature rise of milk cans when heated over steam jets, rises rapidly

to a point where it flattens out sharply as the temperature is reached when there is equilibrium between heat supplied and heat radiated.

10. Milk cans when heated slowly in a steam heated tank type sterilizer, follow the sterilizer temperature closely.

11. Milk cans when heated slowly in a hot air heated tank type sterilizer, lag behind the temperature of the sterilizer to a considerable extent.

PROTEOLYSIS BY STREPTOCOCCUS LACTIS*

L. T. ANDEREGG AND B. W. HAMMER
Iowa State College, Ames, Iowa

INTRODUCTION

The conspicuous change brought about in milk by the growth of *Streptococcus lactis* is the coagulation resulting from the production of acid. The curd formed ordinarily shows nothing suggestive of proteolysis, even after a long period of holding, and in this respect is quite different than the curd produced through the action of the rennin-acid streptococci, such as *Streptococcus liquefaciens*. However, the appearance of a milk culture does not justify the conclusion that the organism is non-proteolytic and chemical examinations are necessary before what seem to be negative results can be accepted.

Certain investigators have reported data showing the decomposition of the protein of milk by *S. lactis* and closely related organisms, while others have failed to detect any such change. In some instances where proteolysis has been noted, the results have not been constant for a given species.

SIGNIFICANCE OF PROTEOLYSIS BY S. LACTIS

Because of the large numbers of *S. lactis* organisms present in certain dairy products, the possibility of proteolytic action due to them is of considerable significance. In the ripening of hard cheeses, for example, the decomposition of the protein is looked upon as being very important and, with the myriads of *S. lactis* organisms that develop during the early stages of the process, these microörganisms may logically be suspected of attacking the protein. The exact factors responsible for those characteristics of butter, cheeses, and fermented cream and milk, which to-

---

* Received for publication October 22, 1928.

gether are spoken of as flavors and aromas, are not completely understood and products which naturally result during the process of proteolysis may be involved in the development of either the desirable or undesirable flavors and aromas.

OBJECT

The primary object of the work herein reported was to determine whether or not a demonstrable proteolytic action occurs when various strains of *S. lactis* commonly isolated in Iowa are grown in milk. A number of the cultures used for developing flavor and aroma in butter were similarly studied because *S. lactis* is one of the two important types of organisms present in such cultures.

REVIEW

Both Orla-Jensen (1) and Barthel (2) mention the original statement by von Freudenreich to the effect tht he found marked digestion of casein due to organisms of the *S. lactis* group. While some investigators apparently have been unable to confirm this finding, others such as Orla-Jensen (1) Barthel (2) and Barthel and Sandberg (3) found considerable proteolysis by organisms of this type.

The *S. lactis* cultures showing protein decomposition have been isolated primarily from milk, butter culture, and cheese. In many of the investigations the trials were carried out with milk to which $CaCO_3$ had been added in amounts sufficient to neutralize all, or nearly all, of the acid that could be formed. Frequently the period of holding the cultures was as long as two or three months. Protein decomposition has been noted with *S. lactis* at various holding temperatures.

Spitzer, Parfitt and Epple (4) found that the *S. lactis* culture studied by them caused a slight increase in various groups of protein decomposition products when grown in milk or in a synthetic butter made by adding skim milk to butter fat.

PROCEDURE

The organisms used in the investigation herein reported were isolated from milk, cream, and cheddar cheese obtained from a variety of sources. Subsequent to isolation they were kept active by transferring through a series of tubes of sterile milk. Transfers were usually made twenty-four or forty-eight hours before a trial was begun so as to have vigorous cultures from which to make inoculations. The butter cultures used were those being carried by the Dairy Section of the Iowa Agricultural Experiment Station, either for investigational purposes or as a matter of routine.

Pint milk bottles were employed as containers since these are more easily handled and stored than Erlenmeyer flasks. The thick wall is a disadvantage but by giving a little attention to the rate of heating in the autoclave the breakage is negligible. For the trials in milk, 200 cc. of skim milk were introduced into each bottle and the bottle cotton plugged, weighed and the weight recorded by marking it on a gummed label which was then fastened to the cotton plug. For the trials in which an attempt was made to neutralize some of the acid, pieces of glass and about 8 grams of finely powdered $CaCO_3$ were placed in each bottle before adding the 200 cc. of skim milk.

The bottles of milk were sterilized in an autoclave in lots of about 40. Generally about twenty minutes were consumed in attaining 15 pounds pressure and heating was continued at 15 pounds for about twenty-two minutes. After shutting off the steam, from twenty-five to thirty minutes elapsed before the door could be opened and the bottles removed. When sterilized in this manner the milk was slightly browned.

In trials that were to last only a short time, equivalent amounts of a milk culture were inoculated into the bottles receiving a given organism, while in trials lasting ten days or more, 2 or 3 drops of a culture were transferred to each bottle.

All the cultures were incubated at room temperature and those containing the added $CaCO_3$ were vigorously shaken daily for a week, and thereafter at intervals of several days.

The soluble portion of each bottle was recovered for the analytical work. In the absence of $CaCO_3$, 1 cc. of concentrated acetic acid was slowly added to the contents of each bottle while it was being gently rotated so as to avoid excessive concentration of the added acid. The bottles were then placed in water and heated until the contents reached 60°C., after which they were removed and cooled. Water was then added to each bottle until the original weight was reached. The bottles containing carbonate were made up to 1 gram less than the original weight and then, with very little agitation, as much as possible of the milk was decanted into another bottle, care being taken to exclude glass and carbonate; 1 cc. of concentrated acetic acid was then added and the heating and subsequent cooling carried out as described. In filtering, the entire lot of milk was thrown on the filter paper at one time and the first filtrate through then returned for a second filtration because of the turbidity. There was some variation in the turbidity of the different filtrates but attempts to more completely clarify them were not successful. Often in duplicate determinations the one giving the clearer filtrate contained just as much soluble nitrogen as the more turbid one; it should not be inferred, however, that such a condition invariably prevailed.

For the nitrogen determinations, 25 cc. of the filtrate were transferred to a 500 cc. Kjeldahl flask and digested with 25 cc. concentrated sulphuric acid, about 5 grams of either sodium or potassium sulphate, a small piece of copper wire and about 2 grams of trichloracetic acid. There was usually considerable foaming but the trichloracetic acid and slow heating until the water was evaporated very materially lessened this. The ammonia was distilled into fifth normal sulphuric acid and the back titrations made with tenth normal sodium hydroxide, using alizarin as an indicator.

The amino nitrogen determinations were made according to the well known Van Slyke method, using 10.0 cc. of sample and retarding the foaming by the addition of a small, measured amount of butyl alcohol.

The lactose was determined by the official general gravimetric method.

Since preliminary trials showed that very satisfactory duplicate determinations were the rule when material from the same filtrate was used, it was considered advisable to inoculate the cultures in duplicate and then run only one determination on each culture. The results, therefore, represent the average of two determinations made on two different filtrates.

The values for the percentage of increase in the soluble nitrogen are sometimes negative; these represent instances in which there was less soluble nitrogen in the filtrate from milk in which the organisms had developed than in the filtrate from the same lot of milk in which there had been no growth. Some work has been done in an attempt to secure an explanation of this, but the exact reasons are not clear.

RESULTS OBTAINED

1. Preliminary trials. The preliminary studies on the proteolytic action of *S. lactis* were carried out using incubation periods of ten, thirty and sixty days and data illustrating the general trend of the results are given in table 1; two trials with butter culture 122 are also included.

Without $CaCO_3$ a number of the *S. lactis* cultures gave definite proteolysis, while others yielded negative values; butter culture 122 gave definite proteolysis in both of the trials in which it was used. In milk to which $CaCO_3$ had been added, definite proteolysis occurred with a number of the *S. lactis* cultures and with the butter culture, while with some of the *S. lactis* cultures it did not. In general, when proteolysis occurred with $CaCO_3$, it also occurred without, but the proteolysis was regularly greater in the presence of the neutralizing agent. When proteolysis occurred, the percentage of the nitrogen in a soluble form ordinarily increased with an increase in the incubation period, but the protein decomposition was quite evident after an incubation of ten days.

There was some variation in the results obtained with a certain culture in different trials. *S. lactis* 1 gave definite proteolysis in trial I but not in trial IV, while *S. lactis* 2 gave proteolysis in trial II but was negative in trial IV.

2. Comparison of the soluble nitrogen increase by lactic acid and by butter cultures. Since the growth of *S. lactis* in milk is inseparably associated with the formation of lactic acid, there is the question as to whether or not the acid so produced is responsible for all or part of the change observed in the soluble nitrogen. In order to get some information on this point two trials were

TABLE 1

Proteolysis by S. lactis and butter cultures in ten, thirty and sixty days

NUMBER OF TRIAL	CULTURE NUMBER	PERCENTAGE INCREASE IN SOLUBLE N					
		No $CaCO_3$ added			$CaCO_3$ added		
		10 days	30 days	60 days	10 days	30 days	60 days
I	*S. lactis* 1	1.3	2.0	2.3	8.1	11.7	12.7
I	*S. lactis* 3	2.9	5.0	2.9	10.3	13.8	14.3
I	*S. lactis* 4				−0.1	0.6	3.9
I	*S. lactis* 7	−1.5	−0.8	−2.1	−0.9	0.4	2.9
II	*S. lactis* 2	3.8	4.1	4.7	7.8	9.9	
II	*S. lactis* 6	−1.0	−1.2	−1.3	0.4	0.6	
II	*S. lactis* 8	−0.7	−0.7			4.9	
II	*S. lactis* 10	1.2	1.5	1.8		4.5	
III	*S. lactis* 5	−1.7	−1.5	−0.9	−0.7		−0.4
III	*S. lactis* 9	−2.1	−1.8	−1.0	−0.6	0.5	0.6
III	*S. lactis* 11	−2.0	−1.9	−1.4	−0.3	0.2	0.1
III	*S. lactis* 14	−1.6	−1.6	−1.1	−0.2	0.4	0.1
III	Butter culture 122	3.3	5.2	6.8	10.4	15.7	21.6
IV	*S. lactis* 1	−2.2	−1.2	−1.4	0.2	−2.0	0.4
IV	*S. lactis* 2	−1.9	−1.3	−1.1	−0.4	−2.0	0.4
IV	*S. lactis* 3	1.8	3.4	4.5	6.5	10.3	13.0
IV	*S. lactis* 4	−1.7	−1.9	−1.3	0.1	−0.4	
IV	Butter culture 122	1.5	3.3	4.6	10.0	12.8	18.2
V	*S. Lactis* 5	−0.3	−0.9		−1.0	0.5	
V	*S. lactis* 6	−1.7	−1.4		0.4	0.5	
V	*S. lactis* 7	−1.5	−1.0		−0.2	0.2	
V	*S. lactis* 8	−1.2	−0.9		−0.1	0.5	
V	*S. lactis* 9	−1.2			−0.1	1.2	

TABLE 2

Comparison of soluble nitrogen increase by lactic acid and by butter cultures

NUMBER OF TRIAL	ACIDITY FROM	PERCENTAGE OF TOTAL N IN SOLUBLE FORM			
		1 day	2 days	3 days	5 days
I	Acid A	12.0	10.9	10.9	
I	Acid B	12.2	10.8	10.8	
I	Butter culture 185	15.3	14.0	14.3	
I	Butter culture 122	15.9	14.9	14.7	
II	Acid A	10.9	10.8	11.3	11.2
II	Acid B	11.1	10.9	11.2	11.2
II	Butter culture 103	14.4	15.0	15.3	15.7
II	Butter culture 146	14.6	15.2	15.4	15.8
II	Butter culture 146–1	14.3	14.7	15.2	14.9
II	Butter culture 122	14.9	14.9	15.0	15.6

carried out in which added sterile lactic acid was compared with acid developed by butter cultures, no $CaCO_3$ being used. Two samples of lactic acid were employed, *Acid A* which had been on hand for a year or more, and *Acid B* which had just been received. After standardizing by titration, the acids were made up to suitable volumes, sterilized and added to the milk in quantities sufficient to give final acidities of 0.8 per cent. The results obtained after different incubation periods are presented in table 2, the data being expressed on the basis of the percentage of the total nitrogen that was in a soluble form. The data show that the amount of soluble nitogen found in the filtrates from each of the butter cultures was always definitely greater than that found in the filtrates from the milk to which the lactic acid had been added. Apparently the addition of lactic acid to the extent of 0.8 per cent did not appreciably proteolyze sterilized milk at room temperature; it, accordingly, appears that the lactic acid produced in milk by the microörganisms of the *S. lactis* group is not responsible for the change in the soluble nitrogen which accompanies their growth in milk.

It is of interest to note that in these trials most of the proteolytic effect of the butter cultures occurred during the first day of incubation.

3. Action of certain cultures on milk with different solid contents. Because of the variations that occurred with certain organisms on different lots of milk, an attempt was made to determine whether there were noticeable differences in the proteolytic action of cultures on milks which differed appreciably in total solids. Two lots of milk were used and were designated as *Milk A* and *Milk B.* The following data briefly show the differences between the whole milk and skim milk from the two lots; only the skim milk was used in the proteolysis trials.

	WHOLE MILK			SKIM MILK		
	Fat	Acidity	Total solids	Sugar	Protein	Ash
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Milk A	3.4	0.14	12.16	4.88	2.86	0.81
Milk B	4.5	0.16	13.86	4.61	3.52	0.84

Nine *S. lactis* cultures were used and were incubated in these milks, both with and without $CaCO_3$, for fourteen days, after which filtrates were secured and analyzed. The results obtained are presented in table 3 as the percentage increase in soluble nitrogen, the percentage increase in amino nitrogen and the percentage of sugar fermented. Three cultures (1, 14 and 39) showed no proteolysis in either lot of milk, while five cultures (23, 24, 25, 42 and 47) showed a definite action; with one culture (26), proteolysis was definite with both lots of milk when $CaCO_3$ was added but was questionable without such an addition.

TABLE 3

The action of certain cultures on milk with different total solids contents

CULTURES	MILK A (LOW TOTAL SOLIDS)						MILK B (HIGH TOTAL SOLIDS)					
	No $CaCO_3$ added			$CaCO_3$ added			No $CaCO_3$ added			$CaCO_3$ added		
	Percentage increase in soluble N	Percentage increase in amino N	Percentage sugar fermented	Percentage increase in soluble N	Percentage increase in amino N	Percentage sugar fermented	Percentage increase in soluble N	Percentage increase in amino N	Percentage sugar fermented	Percentage increase in soluble N	Percentage increase in amino N	Percentage sugar fermented
S. lactis 1	−0.8	0.3	16.4	−2.1		27.9	−1.4	0.2	10.5	−1.5	0.1	20.5
S. lactis 14	−0.9	−0.1	15 9	−0.6	−0.1	33.4	−1.4	0.2	13.1	−1.1	0 1	35.1
S. lactis 23	5.1	1.7	17.6	12.0	3.8	53.6	3.4	2 0	14.6	8.7	4.5	56.8
S. lactis 24	7.1	2.2	15 2	11.3	4.3	32 2	5.5	2.2	13.5	10.5	5 7	55.1
S. lactis 25	1.2	0.6	16.2	5.0	3.0	23.4	1.6	0.8	14.6	4 5	2.9	53.8
S. lactis 26	0.6	0.1	16.8	5.3	2.1	49.4	0.4	0.3	14.6	4.5	2.8	46.0
S. lactis 39	−0.7	−0.1	12.8	−0 2	1.1	47.9	−1.0	0 0	12.9	−1.0	1 3	40 3
S. lactis 42	3.0	0.7	16.4	7.6	2.4	48.5	4.5	1.1	14.6	4.8	3.6	53 9
S. lactis 47				7.1	1.7	46.9	1.4	0.5	16.2	4.8	2.5	45.2

In general, the values for the percentage increase in soluble nitrogen were somewhat less in the milk with a high total solids than in the milk with a low total solids, but the differences are small and are probably explained by the greater amount of protein to be decomposed with the higher total solids.

The data show that a definite increase in the percentage of soluble nitrogen was quite regularly accompanied by a more or less proportional increase in the percentage of amino nitrogen. This is of interest since it shows that some of the protein decomposition procedes to at least the amino acid stage.

The percentage of lactose fermented varied from 10.5 to 17.6 per cent when no $CaCO_3$ was added and from 20.5 to 56.8 when $CaCO_3$ was added. With no added $CaCO_3$, the percentage of sugar fermented was usually lower in the high solids milk than in the low solids milk but, with added $CaCO_3$, there was considerable variation; no definite difference would be expected because of the small difference in the sugar content of the two lots of milk.

4. Influence of the addition of peptone to milk. The influence of the addition of 0.3 per cent peptone to milk on the percentage increase in the soluble nitrogen and in the percentage of lactose fermented was studied using four *S. lactis* cultures and three incubation periods; the results are given in table 4. *S. lactis* 24 was the only culture which gave a definite proteolysis and, with it, the peptone appears to have retarded the protein decomposition. With the three organisms showing no proteolysis, the peptone seems, in general, to have increased the negative values. The peptone appears to have increased the lactose fermentation early in the holding period but the final percentage fermented was not definitely influenced by the addition of the peptone.

TABLE 4

The influence of added peptone on the action of S. lactis cultures in milk

CULTURES	PERCENTAGE INCREASE IN SOLUBLE N						PERCENTAGE OF LACTOSE FERMENTED					
	40 hours		60 hours		14 days		40 hours		60 hours		14 days	
	No peptone	Added peptone	No peptone	Added peptone	No peptone	Added peptone	No peptone	Added peptone	No peptone	Added peptone	No peptone	Added peptone
S. lactis 4	−0.6	−1.9	−0.5	−2.1	−1.3	−4.6	6.0	12.4	11.0	14.4	18.4	18.6
S. lactis 13	−0.5	−2.0	−1.2	−2.0	−1.9	−4.3	6.0	12.8	10.6	16.6	17.4	19.0
S. lactis 24	0.9	0.5	4.2	2.9	6.3	2.8	5.6	11.8	15.6	15.9	16.0	17.0
S. lactis 86	−0.4	−1.3	−1.8		−2.1	−3.9	8.6	11.6	8.0	11.0	15.8	16.4

5. Proteolytic action of a series of S. lactis cultures. Since the *S. lactis* cultures studied were not consistent in their action on

TABLE 5

Comparison of the proteolytic action of various S. lactis cultures

NUMBER OF CULTURE	SOURCE OF CULTURES	GENERAL RATE OF COAGULATION	PERCENTAGE INCREASE IN SOLUBLE N	
			No $CaCO_3$ added	$CaCO_3$ added
		days		
1	Untreated milk	2	−1.2	−0.5
2A	Untreated milk	1	2.1	5.2
2B	Untreated milk	1	3.8	7.0
3	Filtered milk	2	−1.4	−0.2
4A	Untreated milk	1		9.0
4B	Untreated milk	1	6 2	11.0
5A	Commercial butter culture A	1	3.0	2.4
5B	Commercial butter culture A	1	5.7	9.7
5C	Commercial butter culture A	1	1.7	2 0
6A	Commercial butter culture B	1	5.8	10.4
6B	Commercial butter culture B	1	0.9	4.5
6C	Commercial butter culture B	1	−1 2	0 9
7A	Sour cream	1	−1.3	0.3
7B	Sour cream	1	−1.5	0.4
8A	Sour cream	1	2.0	5.4
8B	Sour cream	1	2 0	5.2
8C	Sour cream	1	1.7	7.3
9A	Sour cream	1	2.7	9.1
9B	Sour cream	1	3.5	10.8
9C	Sour cream	1	2.9	7.7
10A	Sour cream	1	2.8	11.5
10B	Sour cream	2	−1.5	0.1
11A	Sour cream	1	5.8	17.3
11B	Sour cream	1	5.7	17.8
11C	Sour cream	1	2.5	12 8
12A	Sour cream	1	2.6	13.2
12B	Sour cream	1	2.7	12.5
12C	Sour cream	1	2.3	11.1
13A	Untreated milk	1	3.4	12.2
13B	Untreated milk	1	3 5	12.7
13C	Untreated milk	1	3.9	12.4
14A	Clarified milk	1	3.1	10.8
14B	Clarified milk	1	3.3	11.0
14C	Clarified milk	1	3.3	12.3
15A	Clarifier slime	1	3.8	5.5
15B	Clarifier slime	1	3.3	5.0
15C	Clarifier slime	1	3.7	5.7
16A	Sour cream	2	0.7	−0.3
16B	Sour cream	2	0.7	

TABLE 5—*Continued*

NUMBER OF CULTURE	SOURCE OF CULTURES	GENERAL RATE OF COAGULATION	PERCENTAGE INCREASE IN SOLUBLE	
			No $CaCO_3$ added	$CaCO_3$ added
		days		
17A	Sour cream	1	4.7	12.6
17B	Sour cream	1	4.6	13.2
17C	Sour cream	1	4.6	13.1
18A	Clarified milk	1	3.4	12.0
18B	Clarified milk	2	−1.2	1.7
19A	Untreated milk	1	4.4	12.7
19B	Untreated milk	1	4.2	13.3
19C	Untreated milk	1	3.6	11.2
20	Untreated milk	4	−0.3	1.3
21	Filtered milk	4	−0.3	0.7
22A	Sour milk	2	−1.2	0.7
22B	Sour milk	1	3.4	11.5
23A	Sour cream	4	−0.6	−0.2
23B	Sour cream	2	3.4	7.0
23C	Sour cream	1	3.1	12.0
24A	Sweet cream	2	3.3	7.8
24B	Sweet cream	1	2.7	6.1
25	Untreated milk	3	−1.4	−0.4
26	Clarified milk	1	1.8	6.0
27	Clarified slime	1	2.2	9.1
28	Clarified milk	4	−1.1	−0.1
29A	Untreated milk	1	2.5	7.0
29B	Untreated milk	1	3.8	7.7
30A	Clarified milk	1	3.8	8.2
30B	Clarified milk	1	4.1	7.7
31A	Clarifier slime	2	3.3	7.6
31B	Clarifier slime	3	−0.6	−0.2
32A	Untreated milk	1	2.0	6.0
32B	Untreated milk	1	2.3	6.6
33	Untreated milk	3	−0.1	0.6
34A	Untreated milk		−0.6	10.5
34B	Untreated milk	1	3.0	8.4
35A	Sour cream	1	2.9	5.7
35B	Sour cream	1	2.8	7.8
36A	Cheese	1	3.5	6.7
36B	Cheese	1	2.8	7.4
36C	Cheese	1	2.2	6.5
36D	Cheese	2	2.8	8.7
37A	Sour cream	1	0.8	3.3
37B	Sour cream	1	1.5	5.0

TABLE 5—*Concluded*

NUMBER OF CULTURE	SOURCE OF CULTURES	GENERAL RATE OF COAGULATION	PERCENTAGE INCREASE IN SOLUBLE N	
			No CaCO₃ added	CaCO₃ added
		days		
38A	Sour cream	7	−0.5	−0.6
38B	Sour cream	7	−1.2	
38C	Sour cream	2	3.2	7.7
39A	Cheese	7	−1.0	−1.0
39B	Cheese	Not curdled in 2 weeks	−0.8	−0.3
39C	Cheese	1	2.2	
40	Cheese	7	−1.0	−0.6
41	Untreated milk	7	−1.4	−0.6
42	Butter culture	1	2.2	3.9

the protein of milk, it seemed advisable to study a considerable number of additional cultures. The cultures used were isolated from untreated, filtered and clarified milk, clarifier slime, sweet and sour cream, butter culture, and cheese. The proteolytic action was tried out, both with and without $CaCO_3$, using fourteen days incubation at room temperature. The results obtained, together with the general rate of coagulation, are given in table 5. Each source is designated by a number and the different letters indicate different isolations from the source.

The data show that some of the cultures caused a very definite proteolysis, while others did not. A comparison of the action of each organism with its rate of coagulation shows that, in general, the cultures which caused a definite proteolysis were the ones which were rapid coagulators, while those causing little or no increase in soluble nitrogen brought about a slow coagulation. In accordance with the results already given, the proteolysis was more extensive when $CaCO_3$ was added than when it was not.

6. Proteolytic action of a series of butter cultures and a series of associated organisms. The proteolytic action of a number of butter cultures from various sources and of a number of the organisms associated with *S. lactis* in butter cultures (*Streptococcus citrovorus* and *Streptococcus paracitrovorus*) was determined, with and without $CaCO_3$, and the results are given in table 6. Incubation was for fourteen days at room temperature. The data show that the butter cultures regularly caused proteolysis, while the cultures of associated organisms did not. With the butter cultures, proteolysis was again regularly increased by the addition of $CaCO_3$ to the milk.

TABLE 6

Proteolytic action of a series of butter cultures and a series of associated organisms

	PERCENTAGE INCREASE IN SOLUBLE N	
	No $CaCO_3$ added	$CaCO_3$ added
Butter culture A	3 0	
Butter culture B	3 5	
Butter culture 103	3 8	9 7
Butter culture 122	3 9	10 8
Butter culture 144	4 2	
Butter culture 146	4 0	9 0
Butter culture 146-1	1 5	
Butter culture 182	2 9	8 3
Butter culture 183	3 5	
Butter culture 185	3 8	
S. citrovorus 5	−0 1	−0 4
S. citrovorus 6	1 1	
S. paracitrovorus 17A	−1 0	−0 5
S. paracitrovorus 2B	0 6	
S. paracitrovorus 2C	−1 4	

DISCUSSION OF RESULTS

The data showing proteolysis with certain *S. lactis* cultures confirm the findings of various investigations along this line. The proteolysis noted with the butter cultures is undoubtedly to be attributed to the *S. lactis* organisms contained since, in the trials carried out, the associated organisms failed to fragment the protein. The increased proteolysis when $CaCO_3$ was added and occasionally distributed by agitation during the fermentation was probably due to an increased growth of the organisms that resulted from keeping down the acidity. Under practical dairy conditions there may be factors which tend to favor proteolysis by *S. lactis* through their influence on the acidity; for example, in old cream held at comparatively high temperatures, the action of molds may reduce the acidity near the surface.

The failure to secure an increase in the soluble nitrogen when sterile lactic acid was added to milk in quantities comparable to those developed by *S. lactis* suggests that the protein decomposition with this organism is due to an enzyme. This enzyme does not show the rapid and extended activity of some of the proteolytic enzymes produced by bacteria, although it does cause a considerable increase in the soluble nitrogen under certain conditions and might easily influence various characters of dairy products, including the flavor and aroma. The increase in the amino nitrogen, as measured by the Van Slyke method, indicates that the protein decomposition goes at least to the amino acid stage. Unpublished results recently secured at the Iowa Agricultural Experiment Station indicate that with certain *S. lactis* cultures the development of ammonia sometimes occurs.

The relationship between protein decomposition and the rate of coagulation suggested by the data presented is of interest because of the variations in the protein decomposition noted by different investigators. The question naturally arises as to whether certain organisms grow slowly because they cannot successfully attack the protein or whether they do not attack the protein because they grow so slowly. Preliminary data which have not been included show that the addition of peptone or certain amino acids to milk into which some of the slow coagulators were inoculated increased the rate of coagulation. This suggests that the organisms may be limited in their growth in normal milk through their inability to satisfy their nitrogen requirements.

The proteolytic action of *S. lactis* may be a factor in various changes that occur in dairy products, such as the protein decomposition in various types of cheese and in butter. The development of flavors and aromas, both desirable and undesirable, in dairy products may be influenced by this proteolysis. The variations in the proteolytic activity of the *S. lactis* organisms present some interesting possibilities in connection with the study of dairy products, especially along the line of the development of butter cultures for special purposes.

CONCLUSIONS

1. Certain cultures of *S. lactis* showed a definite proteolytic activity in milk, while others did not.

2. The butter cultures studied regularly showed proteolysis in milk.

3. When $CaCO_3$ was added to the milk and occasionally distributed by agitation, proteolysis was more pronounced with *S. lactis* cultures and butter cultures than when $CaCO_3$ was not used.

4. Sterile lactic acid added to milk in quantities approximating those developed by *S. lactis* cultures did not increase the amount of soluble nitrogen on holding at room temperature.

5. When there was an increase in soluble nitrogen through the growth of *S. lactis* in milk, there was usually an increase in the amino nitrogen as determined by the Van Slyke method.

6. The addition of peptone appeared to retard protein decomposition rather than accelerate it.

7. In general, protein decomposition with *S. lactis* occurred with cultures coagulating milk rapidly, while no decomposition took place with cultures coagulating slowly.

8. *S. citrovorus* and *S. paracitrovorus* did not cause proteolysis when grown in milk.

REFERENCES

(1) Orla-Jensen 1904 Studien uber die fluchtigen Fettsaüren im Kase nebst Beiträgen zur Biologie der Käsefermente. Centbl. Bakt. 2 Abt., xiii, 514-527.

(2) Barthel, Chr. 1915 Das Kaseinspaltende Vermögen von zur Gruppe Streptococcus lactis gehörenden Milchsaurebakterien. Centbl. Bakt. 2 Abt., xliv, 76-89.

(3) Barthel, Chr., and E. Sandberg 1919 Weitire Versuche uber das Kasein spaltende Vermögen von zur Gruppe Streptococcus lactis gehörenden Milchsäurebackterien. Centbl. Bakt. 2 Abt., xlix, 392-412.

(4) Spitzer, Geo., E. H. Parfitt, and W. F. Epple 1927 A study of the proteolytic action of certain specific organisms on milk proteins in milk and synthetic butter. Jour. Dairy Sci., x, 15-32.

THE EFFECTS OF CERTAIN SALTS ON THE PHYSICAL PROPERTIES OF ICE CREAM MIXES*

J. C. HENING AND A. C. DAHLBERG

New York Agricultural Experiment Station, Geneva, N. Y.

INTRODUCTION

It is recognized that the properties of ice cream mixes may not be uniform from day to day although the mixes appear to have been prepared in the same manner. Different amounts of salts naturally occurring in milk and cream may account for some of the variations in the viscosity of and ease of whipping an ice cream mix. With this idea in mind, the effect of adding sodium or calcium salts, on the viscosity, ease of whipping, and fat globule clumping was determined. Sodium and calcium salts naturally occurring in milk and those used as neutralizers were compared. The calcium in ice cream mixes was precipitated by potassium oxalate and oxalic acid to secure further data on the importance of calcium salts.

EXPERIMENTAL METHODS

The correct proportions of the ingredients in the mix were calculated by the method of Price (1). Cream, skimmilk, powdered skimmilk, water to dissolve gelatin, 0.5 per cent of a medium grade gelatin, and 14 per cent of sugar were the ingredients used in all but four of the experiments. In two experiments butter, skimmilk powder, water, gelatin, and sugar were used and in two other trials skimmilk was used in place of the water. The percentages of fat and serum solids in the finished ice cream were 12 and 10, respectively. The ice cream mixes were pasteurized at 62° to 64°C. (144° to 148°F.) for thirty minutes or at 65°C. (150°F.) for twenty minutes.

From 100 to 200 pounds of mix were prepared for each series of trials. For tests on mixes of normal acidity the former amount

---

* Received for publication October 15, 1928.

was divided into 15 pound lots to which the various salts were added. The salts were always added as a concentrated solution in water to the cold mix before pasteurization and homogenization or to the mix after homogenization as soon as it was cooled. When 200 pounds of mix were prepared half of this quantity was set at 21°C. (70°F.), either with or without the addition of a commercial starter, to develop lactic acid prior to pasteurization. If the starter was added to one portion of the mix an equal amount of skimmilk was added to the other portion. The two portions were divided into 15 pound lots and the same salts were added as before.

The mixes were poured directly into a funnel attached to a Manton-Gaulin homogenizer of 60 gallons per hour capacity. They were processed at the pasteurization temperature and at 2000 pounds pressure per square inch. The mixes were cooled in shot gun cans placed in ice water and were aged at 3° to 5°C. (37° to 41°F.) for fifteen to twenty hours prior to freezing. The mixes were frozen for uniform periods of time in one gallon freezers under identical conditions. The brine temperature was controlled within 0.2°C. and the temperature of the finished ice cream varied half this amount. Some of the results were verified under commercial conditions.

The viscosity of the mix was determined with the MacMichael Viscometer, operated under conditions recommended for the apparatus, using the number 22 and 26 wires which had been standardized by oils of known viscosity furnished by the United States Bureau of Standards. The results obtained were expressed as viscosity in centipoises even though plasticity may have influenced the reading. A more feasible means of determining or stating the fluid characteristics of the mixes was not available. The temperature of the mix, when viscosity determinations were made, varied from 3° to 5°C. but was uniform for each series.

The acidity of the mixes was determined by titration with N/10 sodium hydroxide using phenolphthalein as an indicator.

The fat globules were measured at a magnification of approximately 2000 diameters using an ocular micrometer disc standardized with the microscope so adjusted that each of the smallest marks represented 0.625 micron. One-half cubic centimeter of the mix to be examined was diluted with 100 cc. of distilled water and mounted as a hanging drop preparation.

The ice cream samples were scored for body, texture, and flavor, without knowing their identity at the time of scoring.

EXPERIMENTAL

The effect of salts on viscosity, fat clumping, and ease of whipping

Sommer (2) reported that the ease of whipping ice cream mixes was greatly increased by adding 0.40 or 0.56 per cent of sodium citrate or 1.26 per cent di-sodium phosphate to the mix before aging. Calcium lactate added to the extent of 0.1 and 0.2 per cent had a noticeable effect and 0.5 per cent had a marked effect in reducing the ease of whipping of ice cream mixes. These results have been confirmed in this investigation. In a later article he (3) reported that these results could not always be duplicated.

Sodium and calcium salts naturally occurring in milk and potassium oxalate to precipitate the calcium present in milk were used in the first experiments. Five-tenths of 1 per cent of sodium citrate, potassium oxalate and di-sodium phosphate and 0.2 per cent of calcium lactate were added separately to four portions of an ice cream mix before homogenization, and also to four portions of the same ice cream mix after homogenization.

Table 1 shows the effect these salts had on the viscosity of the ice cream mixes and the percentage of overrun obtained. The sodium salts and the potassium oxalate, added before pasteurization, caused the mixes to be less viscous and whip more readily than the control, whereas the addition of calcium lactate caused the mixes to be very viscous and hard to whip. The addition of 0.5 per cent of calcium lactate caused the mix to curdle excessively as it came through the homogenizer. Even 0.2 per cent caused a small amount of curdling.

The sodium salts added after homogenization did not affect the viscosity of the mixes to any great extent but did improve the ease of whipping. The calcium salts gave practically no effects when added after homogenization.

TABLE 1

Effect on the viscosity and ease of whipping of adding salts to ice cream mixes

EXPERIMENT NUMBER	VISCOSITY IN CENTIPOISES					OVERRUN PERCENTAGE				
	Control	Potassium oxalate	Sodium citrate	Di-sodium phosphate	Calcium lactate	Control	Potassium oxalate	Sodium citrate	Di-sodium phosphate	Calcium lactate
	Salts added before homogenization									
1	3,050	40	43	164	2,020	69	112	110	106	29
2	190	49	54	112	4,680	87	111	100	107	50
3	220	43	41	115	5,010	78	97	101	91	34
4	2,980		1,140		3,850	51		102		30
5	2,130	51	41			74	98	95		46
	Salts added after homogenization									
2	190	179	271	231	242	87	113	103	105	95
3	220	75	350	520	360	78	102	104	85	64
4	2,980		3,400		2,450	51		97		38
5	2,130	370	450			74	97	112		

TABLE 2

Effect on the size of fat globules and fat globule clumps of adding salts to ice cream mixes

SALTS ADDED	NUMBER OF INDIVIDUAL GLOBULES MEASURED	AVERAGE SIZE OF GLOBULES	NUMBER OF CLUMPS MEASURED	AVERAGE SIZE OF CLUMPS
	Salts added before homogenization			
		microns		*microns*
Control	333	1.34	367	5.64 × 3.29
Potassium oxalate	200	1.55		No clumps
Calcium lactate	126	2 03	64	13.08 × 8.33
Sodium citrate	400	1.11	238	2.58 × 1.37
Di-sodium phosphate	100	1.54	100	4.67 × 2.37
	Salts added after homogenization			
Potassium oxalate	80	1.47	54	2.5 × 1.37
Calcium lactate	160	1.46	221	5.76 × 3.27
Sodium citrate	200	1.53	220	5.63 × 3.02

Table 2 gives the average size of individual fat globules and fat globule clumps taken from measurements made in several of the experiments. These are representative of what was found on other slides examined. The mixes to which sodium salts had been added before pasteurization and homogenization contained smaller fat globule clumps. When calcium lactate was added to mixes before pasteurization and homogenization the viscosity and size of fat clusters were increased and whipping was made more difficult. The potassium oxalate mixes were the only ones which have been found to contain no fat globule clumps. One might venture the assumption that the presence of calcium salts was an essential in securing clusters of fat globules by homogenization for no clumps were found in the mixes free from soluble calcium.

The salts added after homogenization did not affect the fat globule clumping except that a slight decrease in the size of clumps was noticed when potassium oxalate was added.

The results of this investigation should not be interpreted to signify the desirability of using any of these salts commercially but rather to show reasons for variations of the properties of ice cream mixes. The ice cream to which sodium citrate, di-sodium phosphate, calcium lactate or potassium oxalate was added was not as fresh in flavor as the control. The potassium oxalate would be very objectionable in commercial ice cream as it is a poison.

The effect of neutralizers on viscosity, fat clumping and ease of whipping

Bendizen (4) reports a trend toward a reduction in overrun with an increase in naturally developed acid. He found no direct relation between the viscosity of mixes and overrun and between viscosity and acidity in untreated mixes. Sodium hydroxide was found to be preferable to calcium hydroxide since sodium hydroxide only reduced the overrun 8.8 per cent as compared to 16.9 per cent reduction by the use of calcium hydroxide. His experiments show that neutralizing the whole mix just before freezing always increases viscosity. Neutralization of the con-

TABLE 3

Effect on the viscosity and ease of whipping of adding salts and acids to ice cream mixes of normal acidity and to mixes which have been allowed to develop lactic acid

EXPERIMENT NUMBER	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	1	2	3	4
SALT ADDED	TITRATABLE ACIDITY					VISCOSITY CENTIPOISES					OVERRUN PERCENTAGE					VISCOSITY CENTIPOISES				OVERRUN PERCENTAGE			
	Mixes of normal acidity																						
	Added before homogenization															Added after homogenization							
Control................	0.20	0.20	0.21	0.22	0.235	76.0	270 0	152 0	578 0	2,130 0	94 7	78 1	84 8	87 0	74 0	76 0	270 0		578 0	94.7	78.1	84.8	87 0
Sodium bicarbonate ..	0.17	0 17		0.21	0.19	63 5	64 0		76 0	161 0	97 0	97 9		102 0	102.0	163 0	296 0		580 0	78.6	75.1		80.2
Calcium hydroxide....	0.16	0 15		0.16	0 20	189 0	289 0		225 0	1,360.0	79 3	75 3		90.3	74 0	177.0	400 0		705 0	73.5	66.2		69.6
Sodium hydroxide.....	0.11	0.12				45 0	49 0				90 0	94 7				304 0	680 0			83 5	81.0		
Citric acid.............	0.31		0 28			7,440 0		6,800 0			46 6		56 2					126 0				98.0	
Oxalic acid	0.295		0.30 0 26			1,140 0		302 0 113 0			80 0		95 8 88 4			68 0		125.0		108.0		96 0	
Lactic acid			0 28					6,500 0					38 0					122 0				95 5	
	Mixes in which acid has been developed																						
Control	0.37	0 27		0.275	0 285	6,300 0	8,100 0		7,350 0	7,460 0	33 4	31 3		43 0	47 7	6,300 0	8,100 0		7,350 0	33 4	31.3		43.3
Sodium bicarbonate ..	0.23	0 26		0 23	0 22	150 0	410 0		380 0	127 0	75 8	80 5		90 6	94 6	5,760 0	6,270 0		5,400 0	38 0	39.5		43.3
Calcium hydroxide ...		0.22		0 23	0 22	1,720 0	7,030 0		3,120 0	560 0	50 0	35 9		52 9	67 5	5,800.0	5,930 0		6,920.0	34 0	25.7		35.9
Sodium hydroxide.....	0.17	0.21				80 0	290 0				90 4	93 0					6,060 0				33.7		

densed skimmilk effected overrun the least and neutralization of the cream the most.

Dahle (5) and Fisher (6) report an increase in viscosity of mixes which are high in acid at the time of homogenization and these mixes could not be whipped to the same overrun as the control. Dahle (5) showed that neutralization improved the ease of whipping but that whipping was not as easy as in the original.

From the preceding results it seemed probable that when sodium salts were used as neutralizers they should produce mixes more easily whipped than when calcium salts were used. Experiments were conducted with the following common neutralizers: sodium bicarbonate, sodium hydroxide and calcium hydroxide. These were dissolved in water and used in quantities calculated as being sufficient to neutralize 0.1 per cent lactic acid. These quantities, expressed on the basis of dry neutralizer, were 0.1 per cent baking soda, 0.04 per cent quick lime, and 0.044 per cent sodium hydroxide. In some trials citric, oxalic, and lactic acids were added to the ice cream mixes, as well as allowing the acidity of certain mixes to increase by natural souring.

In the experiments reported in table 3 neutralizers and acids were added to mixes of normal acidity and to those in which acidity was allowed to develop.

The addition of sodium bicarbonate or sodium hydroxide before homogenization to mixes of normal acidity caused them to whip easier. This was especially noticeable when the control was a little difficult to whip. Their addition after homogenization did not have the same effect.

The addition of lactic or citric acid to ice cream mixes in amounts which increased the titratable acidity to 0.28 to 0.30 per cent caused some curdling when homogenized. These mixes were very viscous and difficult to whip. The addition of the same amount of citric or lactic acid after homogenization did not increase the viscosity of the mix and it whipped very readily. Oxalic acid added in amounts to give a similar titratable acidity either before or after homogenization did not cause the mixes to curdle and did not prevent them from whipping readily.

The mixes in which lactic acid was allowed to develop in amounts which increased the initial titratable acidity to 0.28 to 0.30 per cent curdled some at the time of homogenization, became very viscous, and were difficult to whip. The addition of sodium bicarbonate or sodium hydroxide to these mixes before pasteurization prevented them from becoming unusually viscous and they whipped very readily. When calcium hydroxide was added as a neutralizer the viscosity of the mixes was not reduced as much and they did not whip nearly as readily as those to which sodium bicarbonate or sodium hydroxide had been added. The neutralizers added after homogenization did not reduce the viscosity or improve the ease of whipping.

The low acid mixes to which sodium bicarbonate and calcium hydroxide had been added were very similar in flavor and quality to the control. The flavor of the ice cream containing the sodium hydroxide was slightly pungent.

The ice cream made from mixes which had acid added to them was hard and crumbly but did not taste sour. The ice cream from neutralized mixes in which the acidity was allowed to develop was hard and crumbly and tasted slightly sour. Of the three neutralizers used the best flavored ice cream resulted when sodium bicarbonate was used. The calcium hydroxide did not give as fresh a flavor and the sodium hydroxide gave a neutralizer flavor. The higher the acidity of a mix when it is neutralized the greater the danger of securing neutralizer flavors.

Everyone realizes that the successful manufacture of ice cream of high quality can be effected only through the use of ingredients of high quality. This work shows that when such materials are used and some difficulty is encountered in obtaining the desired overrun the addition of enough sodium bicarbonate calculated to neutralize 0.1 per cent acidity would relieve this difficulty. Likewise when a mix has developed some acidity sodium bicarbonate added before pasteurization will reduce the viscosity, cause the mix to whip easier and improve the flavor of the resulting ice cream.

When mixes were prepared using either unsalted butter, skimmilk powder, and water, or unsalted butter, skimmilk powder, skimmilk, and enough water to mix with or dissolve the gelatin and salts different results were obtained. These results are presented in table 4.

The portions of the mixes in which acidity was increased with a pure culture lactic starter were not as viscous after homogenization as the mixes of normal acidity and they whipped to a little higher overrun. These mixes were all unusually viscous yet they whipped very readily. This is contrary to practically all the other results and should be studied further before a positive statement can be made on this particular point. The control whipped so well that the addition of sodium bicarbonate, or sodium citrate did not improve the ease of whipping. The addition of calcium lactate, however, made them more difficult to whip. Mention should be made of the fact that the control mixes with high acid curdled during processing. The control mix with a titratable acidity of 0.56 per cent was warmed to 100°F. and homogenized. This mix was curdled and could not be heated to pasteurization temperature before homogenization. It was possible to warm the neutralized portions of this mix to

TABLE 4

Effect on the viscosity and ease of whipping of adding salts to ice cream mixes prepared with butter, skimmilk powder and water or skimmilk

EXPERIMENT NUMBER	1	2	3	4	1	2	3	4	1	2	3	4
SALTS ADDED	TITRATABLE ACIDITY				VISCOSITY CENTIPOISES				OVERRUN PERCENTAGES			
	Mixes of normal acidity											
Control	0 22	0 20	0.22	0 24	6,000	4.400	1,790	1,335	90	87	99	105
Sodium bicarbonate	0 16	0 19	0.18	0.18	1,885	2,980	970	612	97	93	107	101
Calcium hydroxide	0 14	0.15	0 16		2,770	4,790	1,810		90	79	98	
Sodium citrate	0.18	0.20	0.21		490	852	332	332	107	102	104	97
Calcium lactate	0 235	0 23	0 25	0 24	8,880	8,900	4.870	3,960	34	47	54	82
	Mixes in which acid has been developed											
Control	0.30	0 25	0.28	0.56	2,590	3,110	2,330	9,300	104	115	109	101
Sodium bicarbonate	0.26	0.25	0 23	0 25	1,745	2,620	1.680	8,700	106	107	116	94
Calcium hydroxide	0 23	0 23	0 22	0 33	1,750	2,450	2,270	11,430	100	105	100	44
Sodium citrate	0 27	0.27	0.28	0 47	435	465	380	1,450	101	98	108	94
Calcium lactate	0.31	0.28	0 28		6,130	3.500	6,680		80	84	61	

62.5°C. (145°F.) before homogenization without excessive curdling. These mixes all bothered in passing through the homogenizer and a constant pressure could not be maintained. A number of uncontrolled factors might cause variations in this particular experiment.

The control prepared with fresh unsalted butter, skimmilk powder, and water was not fresh in flavor. The sodium bicarbonate and calcium hydroxide gave them a little fresher flavor. The sodium citrate produced a salty flavor in the ice cream. The ice cream containing calcium lactate was always poor due to the low overrun obtained. Those mixes which were high in acidity all had a very peculiar sour flavor. The sodium bicarbonate and calcium hydroxide did not counteract this flavor completely but brought about some improvement.

SUMMARY AND CONCLUSIONS

The addition of sodium citrate, potassium oxalate, (poisonous) and di-sodium phosphate to ice cream before pasteurization and homogenization reduced their viscosity and caused them to whip easier. The size of the fat globule clumps in these mixes were smaller. There appeared to be a relationship between the size of fat globule clumps and viscosity. Calcium lactate increased the viscosity and size of fat clusters and made it more difficult to whip. The addition of these salts after homogenization did not have a marked effect on viscosity or size of fat globule clumps but in some cases did affect the ease of whipping the mixes.

A natural increase of lactic acid to approximately 0.30 per cent or the addition of lactic or citric acid to ice cream mixes before pasteurization and homogenization caused them to curdle slightly at the time of homogenization and become very viscous and difficult to whip. The ice creams made from these mixes were hard and crumbly. Oxalic acid added either before or after homogenization or citric or lactic acid added after homogenization did not have this effect. The exceptional action of oxalic acid was attributed to the observed precipitation of calcium.

The addition of sodium bicarbonate and sodium hydroxide to mixes of normal acidity caused them to be less viscous and to whip easier than the control. Calcium hydroxide did not have this effect. Sodium bicarbonate and sodium hydroxide in comparison with calcium hydroxide when used to neutralize ice cream mixes with a titratable acidity of 0.28 to 0.30 per cent produced less viscous and easier whipping mixes. Sodium bicarbonate produced a better flavored ice cream than that produced by the other neutralizers.

The mixes prepared with unsalted butter, skimmilk powder, skimmilk or water were unusually viscous but they all whipped very easily. These mixes, high in acid when pasteurized and homogenized, whipped even better than those of normal acidity. They whipped so well the sodium salts had no effect in this respect but the calcium lactate reduced the ease of whipping.

As a result of these studies it appears probable that the relative amounts of sodium and calcium salts in the ice cream mix made from normal dairy products varies sufficiently to affect the whipping properties of the mix. The mixes prepared from dairy products secured from the same source of supply are not entirely uniform, but when difficult whipping was encountered it was uniformly corrected and a less viscous mix resulted by the addition of 0.1 per cent baking soda prior to pasteurization.

REFERENCES

(1) Price, W. V. 1925 An algebraic method of proportioning ice cream mixes. Jour. Dairy Sci., ix, 243–250.

(2) Sommer, H. H., and Young, D. M. 1926 Effect of milk salts on the whipping ability of ice cream mixes. Jour. Ind. and Eng. Chem., xviii, 865.

(3) Sommer, H. H. 1926 A new factor on the whipping ability of ice cream mixes. Ice Cream Rev., x, no. 3, 134.

(4) Bendixen, H. A. 1925 Do not neutralize ice cream. The Ice Cream Trade Jour., xxi, no. 4, 39.

(5) Dahle, C. D. 1926 The acidity question. The Ice Cream Trade Jour., xxii, no. 12, 49.

(6) Fisher, R. C. 1924 Experimental studies in ice cream during the past year. The Creamery and Milk Plant Monthly, xiii, no. 4, 89.

METALS IN DAIRY EQUIPMENT

METALLIC CORROSION IN MILK PRODUCTS AND ITS EFFECT ON FLAVOR*

O. F. HUNZIKER, W. A. CORDES, AND B. H. NISSEN

Blue Valley Creamery Company, Research Laboratories, Chicago, Illinois

At the present stage of dairy development, contact of milk with metal surfaces in dairy equipment appears inevitable. Milk is exposed to metal surfaces from the time of its production, through the various stages of handling and manufacture, to the packing of the finished product for market. Experience in the use of diverse metals in milk work has demonstrated marked differences in suitability of metals for dairy equipment. These experiences have convincingly shown that suitability of metals is not confined alone to those properties that deal with structural features, heat conductivity and durability, but that it demands serious consideration also of the relation of metals to the flavor and keeping quality of the milk product.

The status of metals in dairy equipment, their properties, resistance to corrosion, and their effect on flavor and keeping quality of dairy products, was brought before the World's Dairy Congress in 1923 by Seligman (1) and Hunziker (2). The past five years have seen new metals and alloys introduced, and additional information has become available on the prominent rôle which corrosion may assume in the life of the equipment and in the maintenance of desirable flavor, keeping quality, and wholesomeness of the dairy product.

It is not the purpose of this discussion to review in detail the theories of metallic action and the plant experiences brought out in the above papers. The present paper deals with the results of

---

* Received for publication October 19, 1928.

specific experiments on corrosion of metals, plated metals, and metallic alloys in contact with diverse milk products and the effect of such contact and corrosion on the flavor of the milk product. In interpreting these new results, however, it is important to take cognizance of the more fundamental facts and theories advanced in the earlier papers above referred to which may be briefly summarized as follows:

In order for any metal to suffer corrosion or to affect the dairy product it must be capable of entering into solution. Such solution may result from the attack of acids, mineral salts, and other constituents of milk as well as from contact with washing powders, chemical sterilizers, brine, etc.; or it may be caused by electrolysis set up in the product itself, or by stray electric currents from imperfectly insulated electrical equipment or conduits within the milk plant or in close proximity. Air has been found to intensify corrosion, making the effect at the air-liquid line and similar points of air contact particularly marked. Injury to the flavor and keeping quality of the dairy product may be caused by the presence, even in very minute amounts, of the metallic salts themselves which have a metallic, bitter, puckery flavor; or by oxidative or catalytic action of these salts, or by bacteria-selective influence of certain metallic salts. Furthermore, it was shown that the salts and oxides of certain metals have distinct toxic properties rendering the product unsafe for consumption.

EXPERIMENTAL

This experiment embraces a study of the corrosive effect of milk and milk products on the various metals used in dairy equipment and the effect of such metals on the milk product itself. This involves extensive laboratory tests, and observations in commercial plant operation.

Materials used. The laboratory tests included 19 different metals, plated metals, and metallic alloys. These metals were used in the form of strips 4 inches long and 1⅝ inches wide, and were as follows:

Single metals

Aluminum*
Copper
Iron
Nickel
Tin
Zinc

Plated metals

Galvanized iron
Tinned copper
  Edges bare§
  Edges tinned**
Copper Retin‡
Tinned iron
  Edges bare††
  Edges tinned‡‡
Manganese aluminum alloy (Al. plated)*

Metallic alloys

Allegheny Metal†
Ascoloy†
Enduro Type "A"§§
Mang. aluminum alloy*
Monel Metal
Nickel silver

* Furnished by the Aluminum Company of America. The aluminum is the pure metal in a soft condition. The manganese aluminum alloy consists of 1.25 per cent manganese and the balance aluminum.

† Furnished by the Allegheny Steel Company. Ascoloy is an ordinary chromium steel alloy, containing approximately 12 to 16 per cent Cr, 0.5 per cent Si, 0.5 per cent Mn., 0.025 per cent P, and 0.025 per cent S. Allegheny Metal is a special chromium steel alloy, containing 17 to 20 per cent Cr, 7 to 10 per cent Ni, and less than 0.5 per cent Mn, 0.5 per cent Si, 0.025 per cent P, and 0.025 per cent S.

‡ Furnished by the Sorensen Speciality Company. This is copper coated with a tin preparation which can be applied to renew the tinned surface without use of flux. The tin coating was very heavy.

The remaining metals were furnished by J. G. Cherry Company.

§ Represents copper strips the cut edges of which were not tinned.

** Represents copper strips the cut edges of which were tinned.

†† Represents iron strips the cut edges of which were not tinned.

‡‡ Represents iron strips the cut edges of which were tinned.

§§ Enduro Type "A" is an ordinary or straight chromium steel containing 16.5 per cent to 18.5 per cent Cr, 0.5 per cent to 1.25 per cent Si, and less than 0.1 per cent C, 0.5 per cent Mn, 0.03 per cent S, 0.03 per cent P, and 0.25 per cent Ni. Since completing this work our attention was called to other Enduro products, such as Enduro 18–8, a chromium-nickel steel. The present experiment refers to Enduro Type "A" only.

The solutions used consisted of 4 organic acids, 2 mineral acids, sweet and sour milks and creams, and sour cream neutralized, as shown in tables 1 and 3.

Method of procedure. The corrosion tests on the metal strips were conducted by immersing them in the liquids tested to the extent of one-half of their length, making it possible to observe the effect at the air-liquid line. Other strips of the same metals, only 2 inches long, were used for total immersion. Additional short strips were provided with rivets of other metals in order to study the effect of metals with different electrical potentials in contact with one another.

In all cases half-pint Mason jars with glass covers were used. Each jar contained 130 cc. of the liquid under test. This made possible submersion of one-half of the length of the strips in each case. The metal strips were cleaned by washing in a very dilute solution of tri-sodium phosphate. They were then thoroughly rinsed in water, wiped dry, dried in a vacuum oven and carefully weighed. After immersing the strips in the respective liquids the jars containing liquid and strip were sealed and held at the temperatures indicated in table 3. At the end of the test period, the metal strips were removed and again washed, rinsed, dried, and weighed as before.[1] The strips were examined for visible corrosion and the liquids for color, precipitate, and flavor, and in some instances for metallic salts. In the case of complete submersion of the metal strips the procedure was identical with that described above, with the exception that the strips were only one-half the length of those used in partial immersion.

METALS IN ACID SOLUTIONS

The acids used were 1 per cent solutions of acetic, butyric, lactic, hydrochloric, and sulphuric acids, and 0.2 per cent solutions of citric acid. Solutions of such weak organic acids as lactic, butyric, citric, and acetic, usually encountered in milk products, were included in these tests chiefly for the purpose of determining the character and extent of action of the acids themselves, and of thus revealing what acids, if any, are most harmful and to what extent the non-acid constituents and other substances present in the dairy product cause corrosion of the metals.

The two mineral acids—sulphuric and hydrochloric—were included chiefly to determine the limit of resistance to corrosion of the several metals.

Results obtained in acid solutions. The weight losses or gains per metal strip are assembled in table 1. They are expressed in milligrams. The area submerged in the acid solution totaled approximately 5.5 square inches. In some instances there was considerable oxidation of the metal above the liquid. This may at times have tended to partly offset the weight losses due to corrosion of the submerged portion. All increases in weight are indicated by a plus (+) sign.

---

[1] The strips were washed and dried in the above manner, rather than by the use of alcohol and ether, to facilitate removal of curdy material. This procedure proved justifiable as the result of numerous preliminary tests which demonstrated absence of noticeable change in weight.

Table 2 describes the appearance of the strips. In some cases the strips were merely tarnished, in others they were corroded or pitted. The effect on the metal at the air-liquid line was quite pronounced in some instances. Thus many of the iron strips corroded most severely just at the surface of the liquid, and in the case of hydrochloric, sulphuric, and acetic acids the strips were deeply etched at the air-liquid line. On the galvanized iron strips, too, corrosion appeared most pronounced right at the surface of the liquid.

In some of the acid solutions considerable white precipitate was present. This was especially noticeable with tin, tinned copper, and tinned iron immersed in sulphuric, acetic, and lactic acids. In the case of Ascoloy and Enduro a very heavy green precipitate resulted in sulphuric acid. No such precipitate appeared with Allegheny Metal.

The organic acid solutions were tasted for presence of metallic flavor wherever possible. Very definite metallic flavor was found in the solutions exposed to the iron and copper strips, and a metallic flavor could be noted in case of the imperfectly tinned iron. In the solutions containing the galvanized iron and zinc, respectively, the acidity had become pronouncedly less, and a sweetish, metallic character could be noted. A slight metallic flavor also was evident in the case of Monel Metal in citric acid.

Discussion of results shown in tables 1 and 2. As was to be expected, the stronger the acids the more intense their action on the metals. In the case of the organic acids, however, those metals which were attacked by lactic acid were affected almost equally as severely by the other organic acids used, even by weak solutions of citric acid.

These tests gave convincing proof of the great resistance of Allegheny Metal to corrosion. This alloy suffered practically no weight loss nor did it show visible corrosion in any of the acids except hydrochloric. In the weaker organic acids, such as lactic, acetic, butyric, and citric, the ordinary chromium steels, Ascoloy

TABLE 1

Loss in weight of metal strips in acid solutions; partial immersion

METALS	1 PER CENT HYDROCHLORIC ACID, 5 DAYS 70°F.	1 PER CENT SULPHURIC ACID, 5 DAYS 70°F.	1 PER CENT ACETIC ACID, 5 DAYS 70°F.	1 PER CENT BUTYRIC ACID, 5 DAYS 70°F.	1 PER CENT LACTIC ACID, 5 DAYS 70°F.	0 2 PER CENT CITRIC ACID, 5 DAYS 70°F.	1 PER CENT BUTYRIC ACID, 5 HOURS 145°F.	1 PER CENT LACTIC ACID, 5 HOURS 145°F.
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm*	*mgm.*	*mgm.*
Single metals:								
Aluminum	404 5	17 8	0 5	2 1	0 4	0 3+	5 6	3 8
Copper	257 9	50 0	48 3	30 1	26 4	26 0	8 2	5 5
Iron	352 5	314 8	417 4	258 6	261 8	200 0	200 2	202 1
Nickel	28 3	55 5	23 3	5 7	34 4	34 0	3 9	5 7
Tin	91 7	27 8	24 3	0 5+	32 9	32 5	0 3	7 2
Zinc	1,200 0	884 7	83 8	75 7	212 9	88 0	8 9+	14 8
Plated metals:								
Copper, tinned E. B.*	56 9	24 0	44 2	2 1	50 8	57 0	1 2	7 1
Copper, tinned E. T†	142 0	22 2	46 9	23 2	53 1	48 5	7 1	9 0
Copper Retin	89 3	30 3	31 6	2 3	134 4	37 8	0 2	7 4
Iron, galvanized	718 6	701 8	650 7	486 5	467 9	136 1	183 2	486 1
Iron, tinned E. B.*	97 6	65 2	93 8	52 3	51 5	47 4	11 7	11 5
Iron, tinned E. T.†	109 6	55 5	63 8	14 6	47 7	27 0	5 0	17 3
Al. alloy, Al. plated	28 7	15 2	0 6	5 9	0 4	0 0	3 8	2.4
Alloys:								
Ascoloy	955 2	653 7	0 0	0 6	0 0	0 5	0 0	0 3+
Allegheny Metal	24 5	0 3	0 0	0 9	0 0	0 5	0 4+	0 0
Enduro	943 1	639 1	0 8	1 5	0 2	2 0	0 6	0 4+
Monel metal	54 9	38 6	29 8	16 7	24 7	20 0	5 7	5 0
Nickel silver	151 6	41 6	26 0	16 5	5 7	16 5	6 7	3 5
Mn. Al. alloy	589 7	16 3	1.0	1 5	0 7	0 0	5 0	4 2

* E. B. designates that the strips were tinned in such a way as to leave the edges bare. This was to represent a condition of imperfect tinning.

† E. T. designates that the strips were completely tinned, edges and all.

+ indicates gain in weight.

TABLE 2

Appearance of metal strips after immersion in acid solutions; partial immersion

METALS	1 PER CENT HYDROCHLORIC ACID, 5 DAYS, 70°F.	1 PER CENT SULPHURIC ACID, 5 DAYS, 70°F.	1 PER CENT ACETIC ACID, 5 DAYS, 70°F.	1 PER CENT BUTYRIC ACID, 5 DAYS, 70°F.	1 PER CENT LACTIC ACID, 5 DAYS, 70°F.	0.2 PER CENT CITRIC ACID, 5 DAYS, 70°F.	1 PER CENT BUTYRIC ACID, 5 HOURS, 145°F.	1 PER CENT LACTIC ACID, 5 HOURS, 145°F.
Single metals:								
Aluminum	$\frac{t}{ccc}$	$\frac{O.K.}{cc}$	$\frac{t}{O.K.}$	$\frac{O.K.}{c}$	$\frac{O.K.}{c}$	$\frac{O.K.}{c}$	$\frac{t}{c}$	$\frac{tt}{cc}$
Copper	$\frac{tt}{ccc}$	$\frac{t}{cc}$	$\frac{ttt}{cc}$	$\frac{tt}{cc}$	$\frac{t}{cc}$	$\frac{t}{ccc}$	$\frac{tt}{cc}$	$\frac{ttt}{cc}$
Iron	$\frac{cc}{cccc}$	$\frac{c}{cccc}$	$\frac{ccc}{ccc}$	$\frac{cc}{ccc}$	$\frac{ccc}{ccc}$	$\frac{cc}{ccc}$	$\frac{cc}{ccc}$	$\frac{c}{ccc}$
Nickel	$\frac{O.K.}{cc}$	$\frac{O.K.}{cc}$	$\frac{t}{tt}$	O.K.	$\frac{O.K.}{tt}$	$\frac{O.K.}{t}$	$\frac{O.K.}{t}$	$\frac{O.K.}{tt}$
Tin	$\frac{t}{cc}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{t}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{t}$	$\frac{O.K.}{cc}$
Zinc	$\frac{c}{cccc}$	$\frac{c}{cccc}$	$\frac{ccc}{c}$	$\frac{cc}{ct}$	$\frac{O.K.}{c}$	$\frac{t}{c}$	$\frac{cc}{ct}$	$\frac{tt}{c}$
Plated metals:								
Copper, tinned E. B.	$\frac{O.K.}{ccc}$	$\frac{O.K.}{ccc}$	$\frac{t}{cc}$	$\frac{O.K.}{t}$	$\frac{O.K.}{ccc}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{t}$	$\frac{t}{cc}$
Copper, tinned, E. T.	$\frac{t}{ccc}$	$\frac{O.K.}{ccc}$	$\frac{t}{cc}$	$\frac{O.K.}{tt}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{t}$	$\frac{t}{cc}$
Copper Retin	$\frac{O.K.}{ccc}$	$\frac{O.K.}{ccc}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{t}$	$\frac{O.K.}{ccc}$	$\frac{O.K.}{cc}$	$\frac{t}{t}$	$\frac{O.K.}{cc}$
Iron, galvanized	$\frac{c}{cccc}$	$\frac{c}{ccc}$	$\frac{ccc}{ccc}$	$\frac{cc}{cct}$	$\frac{c}{cc}$	$\frac{O.K.}{cct}$	$\frac{c}{c}$	$\frac{c}{ccc}$
Iron, tinned E. B.	$\frac{c}{ccc}$	$\frac{c}{ccc}$	$\frac{cc}{ccc}$	$\frac{c}{c}$	$\frac{O.K.}{ccc}$	$\frac{O.K.}{cc}$	$\frac{O.K.}{c}$	$\frac{t}{cc}$
Iron, tinned E. T.	$\frac{c}{ccc}$	$\frac{c}{ccc}$	$\frac{cc}{ccc}$	$\frac{c}{c}$	$\frac{c}{ccc}$	$\frac{c}{cc}$	$\frac{O.K.}{c}$	$\frac{t}{cc}$
Al. alloy, Al. plated	$\frac{t}{cc}$	$\frac{O.K.}{cc}$	$\frac{t}{O.K.}$	$\frac{t}{O.K.}$	$\frac{O.K.}{c}$	O.K.	$\frac{tt}{c}$	$\frac{O.K.}{c}$

O.K. = no change.

c = slightly corroded, cc = corroded, ccc = heavily corroded, cccc = very heavily corroded.

t = slightly tarnished, tt = tarnished, ttt = heavily tarnished.

The condition of the strip above and below the air-liquid line is indicated by the position of the key letter above or below the dash. Thus, tarnished above and corroded below the air-liquid line is indicated as follows: $\frac{tt}{cc}$, heavily tarnished above and heavily corroded below $\frac{ttt}{ccc}$, etc.

TABLE 2—*Continued*

METALS	1 PER CENT HYDROCHLORIC ACID, 5 DAYS, 70°F.	1 PER CENT SULPHURIC ACID, 5 DAYS, 70°F.	1 PER CENT ACETIC ACID, 5 DAYS, 70°F	1 PER CENT BUTYRIC ACID, 5 DAYS, 70°F.	1 PER CENT LACTIC ACID, 5 DAYS, 70°F	0 2 PER CENT CITRIC ACID, 5 DAYS, 70°F	1 PER CENT BUTYRIC ACID, 5 HOURS, 145°F.	1 PER CENT LACTIC ACID, 5 HOURS, 145°F.
Alloys:								
Ascoloy	t ccc	t ccc	O.K.	O.K.	O K.	O.K.	O.K.	O.K. t
Allegheny Metal	O.K. c	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.
Enduro	t ccc	t ccc	O.K.	O K.	O.K	O K. t	O.K.	t O.K.
Monel Metal	t cc	O K. cc	O.K. c	O.K. c	O K. c	O.K. cc	O.K. c	O K. c
Nickel silver	t ccc	tt cc	ttt cc	cc O K.	c t	O K cc	ttt O.K.	tt t
Mn. Al. alloy	t ccc	O K. cc	t O K.	O.K. c	O K. c	O K.	tt c	O.K. c

and Enduro, resisted the action of the acid nearly as completely as Allegheny Metal. Where there was any noticeable difference in appearance it was limited to a barely perceptible brightening of the surface below the air-liquid line when holding the strips at a certain angle in the light, otherwise there was no evidence of visible corrosion on these three steel alloys. The weight losses here were very small, in only two instances did they exceed 1 mgm.

In sulphuric and hydrochloric acid solutions, however the ordinary chromium steels failed to stand up. They rusted and pitted profusely and lost over 600 mgm. in sulphuric and over 900 mgm. in hydrochloric acid. This represents losses of 110 and 180 mgm., respectively, per square inch. This suggests that when corrosion in these ordinary stainless steel products once starts, it proceeds rapidly. Allegheny Metal, on the other hand, resisted the action of such weak sulphuric acid solutions completely, showing not the slightest discoloration and the loss in weight was only 0.3 mgm. It should be pointed out here, however, that this refers to cold 1 per cent solutions only and does not indicate what might occur in stronger solutions and in the presence of heat. In hydrochloric acid this alloy did suffer attack and some loss in weight, but the action was less intense than in the case of any of the other metals and alloys under investigation.

Next to the chromium steels, aluminum and manganese aluminum alloy resisted corrosion in the cold organic acid solutions best. In the heated solutions, however, the strips of the aluminum products showed more intense corrosion and greater loss in weight. The general tendency seems to be that the aluminum plated alloy resisted action more than the other aluminum products. This was especially noticeable in the appearance of the metal strips. In sulphuric acid the aluminum products also stood up better than any metal outside of Allegheny Metal, although they suffered considerable corrosion and loss in weight. In hydrochloric acid, however, aluminum and manganese aluminum alloy failed to show resistance. They lost about 100 mgm. per square inch. The aluminum plated alloy here again was attacked the least, and ranked second best to Allegheny Metal.

Following the chromium steels and the aluminum group in point of resistance to weak organic acids are nickel, tin, Monel Metal, and nickel silver. Of these four, nickel, closely followed by tin, appeared to be superior to Monel Metal and nickel silver from the standpoint of visible corrosion and tarnishing. This group shows considerably greater loss in weight than the previous groups. It is especially noticeable that nickel and tin lost most heavily in acetic, lactic, and citric acid solutions, but resisted butyric acid considerably better. Monel Metal and nickel silver, on the other hand, suffered material loss in butyric acid but less in lactic and citric acid than nickel and tin. Taking the total losses in all organic acid solutions into consideration, nickel and Monel Metal lost most, tin next, and nickel silver least of this group. In this comparison it was at first thought that the heavier tarnishing of the nickel silver may have been a factor in partly offsetting loss in weight. However, our results with total submersion do not bear out this assumption, as here the weight losses of nickel silver were even less than in the case of half immersion. In the mineral acids the corrosion and loss in weight was generally greater than in the organic acids. Nickel suffered less in hydrochloric acid than in sulphuric acid, while tin, Monel Metal, and nickel silver were attacked more intensely by the hydrochloric acid than by the sulphuric acid.

Falling into the next group we find tinned copper, copper, and tinned iron. The visible corrosion on all of these was much greater than in the previous groups, and the loss in weight had also materially increased. In the case of the tinned iron, some rusting through the tin coating was in evidence, indicating imperfection in the tin coat on the iron. In butyric acid solutions the tinned copper and tinned iron closely followed tin in resistance, i.e., they suffered much less loss in weight and showed less visible corrosion here than in the other organic acids. In the case of copper strips the submerged part even in the weakest organic acid solutions showed marked brightening of the surface, an unmistakable evidence that the copper was severely attacked and going into solution. The portion of the copper above the liquid was tarnished heavily, evidently due to the formation of copper oxides.

To the group showing the least resistance to corrosion in these weak organic acids belong galvanized iron, iron, and zinc. Of these, galvanized iron suffered by far the greatest loss in weight. These three metals showed very severe corrosion in all the acids used. It is of particular interest here that these metals showed very heavy corrosion above the liquid in the case of acetic and butyric acids.

The trend of corrosion in the mineral acids was similar to that in the organic acids, the rate of corrosion, however, being much greater in most cases. As might be expected, zinc, galvanized iron, and iron showed the least resistance and, as previously stated, the ordinary chromium steels, Ascoloy and Enduro, also rusted profusely and lost heavily in weight. In all cases except nickel, the corrosion was much greater in hydrochloric acid than in sulphuric acid.

METALS IN MILK AND CREAM

In the study of the effect of metals in milk and cream, as wide a variety of dairy products as is usually encountered in commercial plant operation was used. With this in mind, fresh sweet milk and cream, sour milks of varying acidities and sour gathered cream, both unneutralized and neutralized were included.

The sweet milk tested from about 3 to 3.5 per cent fat with an acidity of 0.16 per cent acid or below. Several different types of sour milks were chosen, such as ordinary creamery buttermilk ranging in acidity from 0.29 per cent at the churn to approximately 0.85 per cent acid at the conclusion of the metal test; butter culture consisting of fresh whole milk ripened by inoculation with a mixed culture of *S. lactis* and *S. citrovorus* to an acidity of 0.83 per cent; and acidophilus milk consisting of fresh whole milk, inoculated and ripened with a pure culture of *L. acidophilus* to 1.76 per cent acid. In both the butter culture and the acidophilus milk the metal strips were immersed in the original sweet milk and kept in the liquid during the preparation, ripening, and holding of these products. The cream, both sweet and sour, was direct shipper cream averaging approximately 35 per cent fat. The sweet cream tested from 0.11 to 0.14 per cent acid at arrival and 0.16 to 0.25 per cent at the conclusion of the holding period. The sour cream, upon arrival at the factory, tested from 0.5 to 0.6 per cent acid and was then ripened without culture to an acidity of 1 per cent. This was done to simulate severe summer conditions. Two different periods of exposure and temperatures were used, namely five hours at 145°F. and five days at room temperature (70°F.). In the case of neutralized cream, two sets of tests were made. In the first test the original sour cream was neutralized with calcium magnesium lime from 0.5 to 0.15 per cent acid. Then the metal strips were immersed and the cream was held at 145°F. for five hours. In the second set, the metal strips were immersed in the original cream, testing 0.5 to 0.6 per cent acid, just before neutralization. The cream was then neutralized with lime to 0.15 per cent acid, then pasteurized at 145°F. for thirty minutes, cooled to 45°F., and held overnight. The metal strips were then removed, washed, steamed, and the same procedure repeated daily for ten consecutive days. The acidities of the several lots of milk and cream at beginning and end of each test and the times and temperatures of exposure were as shown in table 3.

Tables 4, 5, 6, and 7 show the results obtained in milk and cream, sweet and sour, and hot and cold. Table 4 gives the weight losses and table 5 the visible corrosion of the metal strips; table 6 indicates the effect on the flavor of the dairy product. In table 7 are recorded the results obtained by exposing the metal strips to sour cream during neutralization, pasteurization, cooling and holding, daily for ten consecutive days with a fresh batch of cream each day as previously described under materials used.

Discussion of results shown in tables 4, 5, 6 and 7. In general it was noted that where the metal was able to resist corrosion in weak organic acid solutions it was likewise not seriously affected by milk and cream, and did not produce objectionable flavors in these products. This would indicate that the highly complex colloidal nature of the dairy product apparently did not alter the general resistance of the metal to corrosion by acids. In fact, the corrosion in the cream containing 1 per cent acid was less intense than in the 1 per cent lactic acid solution, suggesting that the non-acid constituents tend to exercise a protective influence.

TABLE 3

Temperature, acidity, and period of exposure of milks and creams

DAIRY PRODUCTS	PERIOD HELD	TEMPERATURE	ACIDITY AT BEGINNING	ACIDITY AT END
		°F.	*per cent*	*per cent*
Sweet milk	5 days	70	0 14–0 15	0 26–0 32
Sweet milk	5 hours	145	0 15	0.15
Sweet cream	5 days	70	0 14	0 25
Sweet cream	5 hours	145	0 14	0 16
Buttermilk	5 days	70	0 29	0 82–0.87
Butter culture	15 hours	70	0 16	0 83
	30 hours	40		
Acidophilus	5 days	98	0 16	1 76
Sour cream	5 days	70	1 00	1 00
Sour cream	5 hours	145	1 00	1 00
Sour cream neutralized	5 hours	145	0 15	0 15
Sour cream neutralized	Factory procedure, daily for 10 days		From about 0.6 to about 0.15	

Effect of milk and cream on the metals. As shown in tables 4, 5, and 7 the corrosion and loss of weight of the metal strips and the

TABLE 4

Loss in weight of metal strips in sweet and sour milks and creams; partial immersion

METALS	SWEET MILK		SOUR MILKS			SWEET CREAM		SOUR CREAM		SOUR NEUTRALIZED CREAM
	70°F., 5 days, acid 0.15 per cent	145°F., 5 hours, acid 0.15 per cent	Acidophilus 98°F., 5 days, acid 1.76 per cent	Butter-culture 70°F., 2 days, acid 0.83 per cent	Buttermilk 70°F., 5 days, acid 0.29 to 0.87 per cent	70°F., 5 days, acid 0.16 per cent	145°F., 5 hours, acid 0.16 per cent	70°F., 5 days, acid 1.0 per cent	145°F., 5 hours, acid 1.0 per cent	145°F., 5 hours, acid 0.15 per cent
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Single metals:										
Aluminum	0.7+	0.1	1.0	0.0	0.1	0.7	0.1+	1.1+	0.6	0.7
Copper	1.1	1.8	4.6	1.9	7.2	4.0	2.4	3.5	3.3	1.0
Iron	24.2	4.6	179.6	62.3	147.6	0.0	5.1	127.6	72.8	2.5
Nickel	2.6	1.2	14.6	4.3	6.4	5.4	2.4	3.9	2.7	0.0
Tin	2.7	0.5	4.4	0.8	2.6	0.3	0.8	1.1	0.5	0.8
Zinc	3.6	0.1	41.1	5.0	21.0	1.2	1.4	18.3	8.8+	5.0
Plated metals:										
Copper, tinned E. B.*	0.4	0.6	4.8	0.6	0.3	1.5	0.3+	1.4	1.6	0.2
Copper, tinned E. T. †	0.0	0.7+	4.3	3.5	3.4	1.0	0.1+	2.8	1.3	0.1
Copper Retin	2.5	0.1	0.9	0.4	1.7	0.3+	0.8	0.5	0.8	1.1
Iron, galvanized	21.2	0.3	447.2	9.6	41.6	9.9+	0.9+	1.0+	17.7	2.4
Iron, tinned E. B.	3.7	0.7+	6.0	2.2	15.7	2.9	0.3	6.4	5.0	1.0
Iron, tinned E. T.	0.3	0.1	4.9	0.8	3.6	1.2	0.1	7.2	3.9	1.8
Al. alloy, Al. plated	0.9+	0.8	1.2	0.0	0.2	0.0	0.5+	1.2+	0.6	0.3

Alloys:										
Ascoloy	0 1	0 9+	1 3	1 4	0 0	0 1	0 6	1 4	0 4	0.4
Allegheny Metal	0 2	0 8+	0 7	1 4	0 0	0 4	0 0	1 3	0 5+	0.4
Enduro	0 0	1 1+	0 7	1 3	0 0	1 3	0 4+	1 0	0 3	0.7+
Monel Metal	1 3	0 5	7 3	1 9	2 2	2 1	0 3	2 8	1.4	0 3
Nickel silver	3 3	1 2	9 9	3 0	3 2	4 1	2 8	5 0	1 9	0 3
Mn. Al. alloy	0 4+	0 5+	1 5	0 4	0 9	1 4	0 5+	0 3+	1 1	0 4

* E. B. designates that the strips were tinned in such a way as to leave the edges bare. This was to represent a condition of imperfect tinning.

† E. T. designates that the strips were completely tinned, edges and all.

+ indicates gain in weight.

TABLE 5

Appearance of metal strips after immersion in dairy products; partial immersion

METALS	SWEET MILK		SOUR MILKS			SWEET CREAM		SOUR CREAM		SOUR NEUTRALIZED CREAM
	70°F., 5 days, acid 0.15 per cent	145°F., 5 hours, acid 0.15 per cent	Acidophilus 98°F., 5 days, acid 1.76 per cent	Butter-culture 70°F., 2 days, acid 0.83 per cent	Buttermilk 70°F. 5 days acid 0 29 to 0.87 per cent	70°F., 5 days, acid 0.16 per cent	145°F, 5 hours, acid 0 16 per cent	70°F, 5 days, acid 1 0 per cent	145°F., 5 hours, acid 1.0 per cent	145°F., 5 hours, acid 0.15 per cent
Single metals:										
Aluminum	O.K.	tt / O.K.	tt / c	O.K.	O.K.	O.K.	O.K.	O.K.	O.K. / c	t / t
Copper	O.K. / cc	ttt / cc	tt / ccc	tt / cc	tt / ccc	t / cc	tt / cc	tt / cc	tt / cc	tt / cc
Iron	c / t	c / t	ccc / ccc	ccc / cc	c / ccc	cc / tt	c / tt	ccc / ccc	ccc / cc	c / tt
Nickel	O.K. / tt	O.K. / tt	O.K. / cct	O.K. / ttt	O.K. / c	O.K. / ttt	O.K. / t	O.K. / t	O.K. / t	O.K. / ttt
Tin	O.K. / t	O.K.	O K. / tt	O K.	t / t	O.K. / t	O.K.	O.K.	O.K. / t	tt / tt
Zinc	c / cc	t / cc	cc / ccc	tt / cc	cc / cc	cc / cc	c / cc	cc / cc	cc / cc	ttt / cc
Plated metals:										
Copper, tinned E. B.	O.K.	t / O.K.	t / c	t / cc	O.K.	c / c	tt / tt	O.K. / c	O.K. / c	t / ct
Copper, tinned E. T.	O.K.	O.K.	t / c	t / c	O K.	t / c	t / O.K.	O.K. / c	O.K. / c	t / ct
Copper Retin	t / c	O.K.	t / O.K.	O.K.	O.K. / c	O.K.	t / O.K.	O.K.	t / O.K.	t / O.K.

Plated metals—continued:										
Iron, galvanized	c/cc	t/c	cc/ccc	cc/c	c/cc	cc/c	c/O.K.	c/cc	cc/cc	tt/cc
Iron, tinned E. B	t/O.K.	t/O.K.	cc/cc	O.K	c/O.K.	t/O K.	O.K./t	c/O.K.	cc/c	t/O.K.
Iron, tinned E. T.	cc/O.K.	O.K.	cc/c	c/c	c/c	O.K./c	O.K./c	c/c	cc/c	c/c
Al. alloy, Al. plated	O K.	O K.	t/c	O.K.	O.K.	O.K.	t/O K.	O.K./t	t/c	t/t
Alloys:										
Ascoloy	O.K.	O.K.	O.K./c	O K.	O.K.	O.K.	O K.	O.K.	O K./t	O.K./t
Allegheny Metal	O.K.	O.K.	O.K.	O.K.	O K.	O.K.	O.K.	O.K.	O.K.	O.K.
Enduro	O.K.	O.K	O.K.	O.K.	O K.	O.K.	O.K.	O.K.	t/O.K.	O.K.
Monel Metal	O.K./ttt	O.K/ttt	O K./ttt	O.K./ttt	O K./tt	O K./t	O.K./tt	O.K./tt	O K./tt	O.K./tt
Nickel silver	t/ttt	t/tt	cc/ttt	tt/tt	tt/ttt	t/tt	t/t	ttt/ttt	ttt/t	tt/t
Mn. Al. alloy	O.K.	O.K.	tt/c	O.K.	O.K.	O.K.	tt/O.K.	O.K./t	t/c	O K./t

TABLE 6

Flavor of sweet and sour milks and creams; partial immersion

METALS	SWEET MILK		SOUR MILKS			SWEET CREAM		SOUR CREAM		SOUR NEUTRALIZED CREAM
	70°F., 5 days, acid 0.15 per cent	145°F., 5 hours, acid 0.15 per cent	Acidophilus 98°F., 5 days, acid 1.76 per cent	Butter-culture 70°F., 2 days, acid 0.83 per cent	Buttermilk 70°F., 5 days, acid 0 29 to 0.87 per cent	70°F., 5 days, acid 0.16 per cent	145°F., 5 hours, acid 0.16 per cent	70°F., 5 days, acid, 1.0 per cent	145°F., 5 hours, acid 1.0 per cent	145°F., 5 hours, acid, 0.15 per cent
Single metals:										
Aluminum			Sl. Met.	Sl. Met.						
Copper	Tallowy	Sl. Met.	Sl. Met.	Metallic	Green Whey	Tallowy	V. Met.	Metallic	Metallic	Sl. Met.
Iron	Metallic	V. Met.	V. Met.	V. Met.	Metallic	V. Met.	V. Met.	V. Met.	V. Met.	Sl. Met.
Nickel			Sl. Met.	Sl. Bitter		Sl. Bitter	Sl. Met.			
Tin										
Zinc			V. Met.	Metallic	Sl. Met.				Sweetish	Metallic
Plated metals:										
Copper, tinned E. B.				Sl. Met.		Bitter	Sl. Off		V. Met.	
Copper, tinned E. T.						Bitter				
Copper Retin										
Iron, galvanized	Fishy	Metallic	V. Met.	Metallic	Metallic		Sl. Off	Bitter	Sl Bitter	Metallic
Iron, tinned E. B.			V. Met.	V. Met.	Metallic	Sl. Met.			Metallic	Sl. Met.
Iron, tinned E. T.		Metallic	Sl. Met.	Sl. Met.		Bitter			Metallic	Sl. Met.
Al. alloy, Al. plated				Sl. Met.					Metallic	
Alloys:										
Ascoloy			Metallic							
Allegheny Metal										
Enduro			Metallic							
Monel Metal				Sl. Met.			Off			
Nickel silver		Sl. Met.	Sl. Met.	Metallic			Metallic		Sl. Met.	
Mn. Al. alloy							Sl. Met.			

V. = very; Sl. = slightly; Off = off flavor.

TABLE 7

Neutralized cream treatment simulating factory operation

Strips exposed to sour cream (0.5 to 0 6 per cent) neutralized to 0.15 per cent, pasteurized at 145°F., held thirty minutes, cooled to 45°F., held overnight. Treatment repeated daily for ten consecutive days, using a fresh batch of cream each day.

Total loss from first to last treatment

METALS	AFTER FIRST TREATMENT		AFTER THIRD TREATMENT		WEIGHT LOSS				TENTH TREATMENT	
	Weight loss	Flavor	Weight loss	Flavor	5th	6th	7th	8th	Weight loss	Flavor
	mgm.		*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Single metals:										
Aluminum	0 2	O.K.	0 3	O.K.	1 0	1 3	0 7	0 4	0 7	O.K.
Copper	2 1	Sl. Met.	3 1	O.K.	5 9	7 1	6 9	6 5	7 0	Sl. Met.
Iron	4 0	Met.	23 4	Met.	37 8	44 3	47 7	49 8	59 1	Met.
Nickel	2 1	O.K.	4 6	O.K.	7 0	7 0	7 7	8 4	9 9	O.K.
Tin	0 6	O.K.	1 5	O.K.	1 8	2 1	1.4	1 4	1 9	O.K.
Zinc	2 3	V. Met.	2 5	Sl. Met.	3 0	15 4	18 8	20 7	25 4	Sl. Met.
Plated metals:										
Copper, tinned E. B.	0 4	O.K.	1 0	O.K.	1 3	0 8	1 0	1.1	1 8	O.K.
Copper, tinned E. T.	0 5	O.K.	5 0	Sl. Met.	5 6	5 5	5 5	5 6	6 5	O.K.
Copper Retin	0 5	O.K.	0 6	O.K.	1 4	1 7	1 1	1 0	1 1	O.K.
Iron, galvanized	1 1+	V. Met.	2 1	Met.	44 6	44 1	43 0	44 9	48 4	Sl. Met.
Iron, tinned E. B.	0 6	V. Met.	2 8	Met.	6 1	6 4	5 8	6 5	8 7	Sl. Met.
Iron, tinned E. T.	1 2	O.K.	2 2	O.K.	3 5	4 6	3 4	3 8	4.7	Sl. Met.
Al. alloy, Al. plated	0 4	O.K.	0 0	O.K.	0 5	2 1	0 7	0 4	0 9	O.K.
Alloys:										
Ascoloy	0 0	O.K.	0 6	Sl. Met.	0 7	0 9	0 2	0 4	0 4	O.K.
Allegheny Metal	0 0	O.K.	0 8	O.K.	0 7	0 5	0 1	0 0	0 1	O.K.
Enduro	0.6+	O.K.	1 2+	O.K.	0 5+	0 5+	1 2+	0 7+	0 1+	O.K.
Monel Metal	0 8+	O.K.	0 0	O.K.	0 6	1 3	1 3	1 2	1 4	O.K.
Nickel silver	0 8	Sl. Met.	2 7	O.K.	3 9	3 9	3 8	4 0	5 1	O.K
Mn. Al. alloy	0 4	O.K.	0 5	O.K.	0 7	1 3	0 6	0 4	0 6	O.K.

+ indicates gain in weight.

Met. indicates metallic flavor; Sl. Met., slightly metallic; V. Met., very metallic; O.K., no off flavor.

order of their resistance are in the general direction indicated by the results in weak organic acid solutions as previously given in tables 1 and 2, though the intensity of corrosion in the dairy products is markedly less.

Thus iron, galvanized iron, and zinc again were poorest, and showed the least resistance, as indicated both by the visible corrosion on the strips and by the weight losses recorded, while the chromium steels and aluminum products withstood attack most successfully. Tin and tinned copper also showed comparatively high resistance. Nickel, copper, Monel Metal and nickel silver suffered rather severe tarnishing and considerable loss in weight, particularly in the high acid milk products.

Comparison between sweet and sour liquids indicates, with very few exceptions, more severe action in the sour liquids. This again emphasizes that acidity in milk products is the dominating factor in corrosion directly due to contact with dairy products. But even in the sweet milk products many of the metals showed marked tarnishing and some corrosion. This was particularly noticeable in the case of nickel, nickel silver, and Monel Metal.

It is further interesting to note that in the neutralized sour cream exposed to 145°F. for five hours, tables 4 and 5, all metals showed comparatively high resistance, with the possible exception of iron, galvanized iron, zinc, and copper. In the neutralized sour cream, table 7, in which the metal strips were immersed just before neutralization and the treatment was repeated daily for ten consecutive days, however, the intensity of corrosion was greatly augmented. The attack on iron, galvanized iron, and zinc here again was by far the most severe, but nickel, copper, tinned iron, and nickel silver also suffered very considerable loss in weight. Tin proved relatively resistant. The tinned copper surfaces also held up well, except in one case where the loss was almost equal to that of copper. This suggests that the resistance of the tinned metal is dependent very greatly on the quality of the tinning. This may also help to explain the comparatively heavy loss in weight of the tinned iron strips, due probably to the greater porosity of the tin coating on iron.

The above experiment was conducted in such a way as to expose the metals daily to a new batch of cream for ten consecutive days. The treatment given was similar to that in commercial plant operation. It appears justifiable to assume that these metals would show approximately the same order of resistance in large-scale, commercial equipment.

Effect of the metals on milk and cream. Each sample of cream was carefully graded, especially for metallic flavor, by several independent judges after the strips were removed from the test.

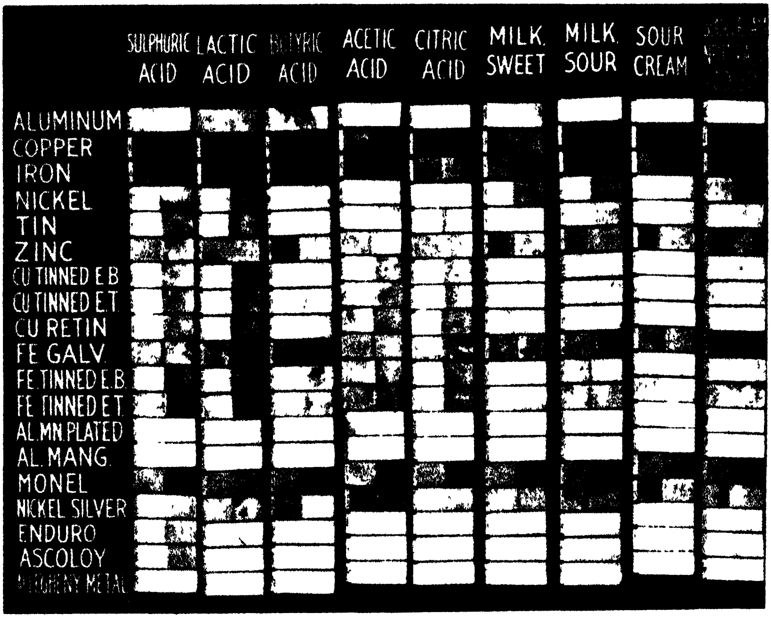


Fig. 1. Sets of Metal Strips after Half Immersion in the Liquids Listed at the Top

These results are shown in tables 6 and 7. In some cases, such as sour cream held at room temperature for five days, other changes had taken place making it very difficult to recognize the metallic taste. Iron, copper, and galvanized iron, however, even here left such extremely metallic flavor that there was no difficulty in readily recognizing them. In all the other milks and creams the metallic or other characteristic flavors due to metallic action could be easily detected. On the whole, however, the results obtained with butter culture were found the most reliable, as even delicate shades of off-flavor seemed to stand out definitely. Thus, in the latter case, i.e., with butter culture, our results show iron and tinned iron with the edges untinned to produce the most decided metallic flavor. Copper, zinc, galvanized iron and nickel silver also gave the butter culture a distinct metallic taste. Aluminum, nickel, copper tinned with edges not tinned, iron completely tinned, manganese aluminum alloy plated, and Monel Metal developed a slight metallic flavor. The remaining metals and alloys, namely tin, completely tinned copper, manganese aluminum alloy, and the chromium steels, failed to have any effect on the flavor of the culture. It will be noted that in the case of this milk product the metal strips remained in the milk during every step in the preparation of the culture, to the end of the test which totaled in all two days, and that the acidity developed during this period from 0.16 to 0.83 per cent.

Because of the outstanding importance of the effect of the various metals on the flavor of the dairy products it is deemed desirable to discuss the results obtained somewhat in detail, grouping the metals according to their action on flavor.

Allegheny Metal, tin, and tinned copper. Allegheny Metal, tin, and completely tinned copper were the only metals that produced no metallic flavor in any of the milk products.

Allegheny Metal, the chromium-nickel steel alloy, showed no visible corrosion in the dairy products and there was practically a complete absence of loss in weight. Where such losses did occur they were invariably exceedingly small and apparently of no significance. In fact, Allegheny Metal ranked best of any of the metals under study. Where there was absence of corrosion in the case of several of the other metals, this alloy invariably also proved entirely immune. In liquids (acid solutions) and under treatment that caused serious corrosion of the other metals, Allegheny Metal was either not affected at all, or led the list in point of resistance to such corrosion. In only one instance did Allegheny Metal show visible attack. This was its exposure to hydrochloric acid and here again the corrosion was much less than that of the other metals, with the exception of aluminum plated alloy and nickel, which lost only slightly more in weight than Allegheny Metal. In no case did Allegheny Metal injure the flavor of the milk product.[2]

This absence of corrosion suggests absence of solution of the metal. Since the development of metallic flavor depends upon solution of the metal and presence of the dissolved metal in the product, the complete absence of metallic flavor in all the milk products tested, i.e., exposed to Allegheny Metal, may be readily explained. These favorable qualities of Allegheny Metal emphasize its high desirability in dairy equipment and place it at the top of the list of metals studied in this experiment. In pointing out these superior qualities of Allegheny Metal, the nickel-chromium steel, attention should be called to the fact that this alloy differs from the ordinary chromium steels, or so-called stainless steels, in that the latter do not contain nickel and usually have a lower content of hromcium. The ordinary chromium steels, Ascoloy and Enduro, while showing high resistance to corrosion in most cases, did suffer slight attack under some conditions and in the case of acidophilus milk actually produced a metallic flavor.

These observations with Allegheny Metal are supported by the work of North (3). An analysis of his figures shows that Allegheny Metal ranked first and better than nickel and Enduro. Quam (4) also compared the resistance of these chromium steels and found Allegheny Metal far superior to the ordinary chromium steels. Grindrod (6), who, before the advent of Allegheny Metal in milk equipment, considered all-nickel and all-tin the only metals suitable, reports that Allegheny Metal has proven fully equal and in some respects superior to nickel in the construction and use of milk condensing and sterilizing equipment. Dahle (7) states that he used an Allegheny Metal tank repeatedly for making pure culture milk with an acidity of 1 per cent and noticed no spots nor discoloration on the metal, nor any off-flavors in the milk. MacQuigg (8) reports that high-chromium steels and chromium-nickel steels showed greater resistance to corrosion in the handling of corrosive chemicals than any other metal or alloy.

---

[2] It should be pointed out here that Allegheny Metal is but one representative of the general group of chromium-nickel-steel-alloys At the inception of this investigation Allegheny Metal was the only product of this group then available to us Other chromium-nickel steels of similar composition have since become available for the fabrication of dairy equipment

It is important, however, to call attention to the fact that Allegheny Metal is vulnerable and may suffer rusting and pitting when in contact, on the liquid side, with certain other metals. The attack here is caused by electrolysis and will vary in character and intensity with the electrical potential of the metal in contact. Users of Allegheny Metal tanks have learned that when such equipment contains rivets, bolts, fittings, or other accessories of such metals as iron, copper, nickel silver, Monel Metal, tin, etc., the resistance of the alloy to corrosion may be materially diminished and rust spots may appear. These findings support our results on multiplicity of metals in which the Allegheny Metal strips with iron rivets and tin rivets tarnished noticeably in some cases. Rusting may also occur on welded surfaces of Allegheny Metal in the absence of proper finishing of the weld, such as will remove all traces of iron oxide that may have formed during the welding process. As will be shown in a later paper, certain treatment with brine also has some effect on Allegheny Metal. Further observations emphasize that the maximum resistance to corrosion depends very much upon a high polish of the surface of the Allegheny Metal and that unpolished surfaces did yield some rusting.

Tin and properly tinned copper, as previously stated under this group heading, also failed to produce metallic flavor in any of the milk products. These metals, however, showed slight corrosion and considerable loss in weight in some instances. The weight losses of tin and properly tinned copper averaged slightly over twice as much as those of the chromium steels.

While the solubility of tin in the milk product is not inconsiderable, the salts of tin appear to cause no noticeable injurious effect on the flavor of the milk product. In the present experiment the pure tin proved entirely harmless in this respect. Under no condition, regardless of temperature, acidity, or time of exposure of the milk product, was there the slightest suggestion of metallic or other objectionable off-flavor. These results are in harmony with the findings of Donauer (17), who shows that while tin is more soluble than copper and copper alloys, it required ten times as much tin in solution as it does copper to produce a distinct metallic flavor. That tin is incapable of inciting progressive decomposition and damage to flavor in butter while, under identical conditions, iron, copper, and nickel silver spoiled the butter entirely, bleaching it and making it intensely tallowy in flavor, was demonstrated by Hunziker and Hosman (9).

Further experimental proof of the action of weak organic acids on tin is cited by Mantell and Lincoln (10). These investigators studied the subject from the standpoint of the food canning industry and found that citric, lactic, and acetic acids attack tin and tin alloys. The life of the tin coating is further shortened by the excessive use of caustic alkali washing powders for cleaning, and hypochlorite solutions used for sterilizing the equipment.

Another possible contributing factor to the short life of the tin coating on copper may be the contamination of the tin with copper due to method of tinning. Thus, Merica (11) points out that in the usual method of tinning copper, the tin bath tends to become contaminated with copper and there is danger of obtaining a brittle tin coating.

All of these facts emphasize the great importance of a proper method of tinning copper and maintaining a complete tin coating on the copper surface. Manifestly, a complete and heavy tin coating of copper provides the most effective protection against the corrosion of copper and its damaging effect on the milk product. Properly tinned copper offers a safe surface for exposure to milk products. Tinned copper furnishes better protection in this respect than tinned iron. The life of the tin coating, however, is relatively short. At best, retinning at frequent intervals, therefore, becomes necessary in order to guard against the damaging effect of exposed copper. Even in such weak organic acid solutions as may be encountered in sweet and sour milk products, such as lactic, butyric, citric, and acetic acids, the corrosion and loss in weight was quite considerable. Tin, furthermore, is very soft so that the loss by friction and wear may be quite an important factor.

It appears pertinent here to suggest that improvement in the method of tinning leading to better contact and greater permanency of the tin coating on the copper surface, and possibly the hardening of the tin itself, are important problems, the satisfactory solution of which would eliminate some of the current objectionable features and would offer additional advantages to the user of tinned copper equipment.

The distinct absence of metallic flavor in dairy products held in contact with tin, together with the physical and fabrication advantages and the comparative ease of retinning, explain the continued popularity of tinned copper surfaces in dairy equipment, although the desirability of the presence in milk products of such metallic salts as the salts of tin may well be questioned.

Nickel. As far as the effect of nickel on the flavor of the milk product is concerned, the present experiment shows that nickel is not entirely negative. While no intense metallic flavor resulted from nickel in any of the milk products, a slight metallic and bitter flavor was noted in the case of acidophilus milk, butter culture, and in sweet cream, hot and cold. The ready solubility of nickel in weak organic acid solutions and in acidophilus milk suggest that nickel equipment is not so suitable for high-acid dairy products as has been generally assumed. In the milk products, table 4, and in the neutralized cream, table 7, the corrosion of nickel resulted in losses that averaged 3 and 5 times greater, respectively, than those for tin. Similar observations were also reported by Cornell (12), in his experiences with milk plants, who concludes that nickel is not so satisfactorily used in connection with the development of high-acid in dairy products. Burrell (5) reports that nickel hoops used for draining Camembert cheese were a failure and actually caused the cheese to turn green. Another manufacturer reported that a large all-nickel surface coil cooler installed in a city milk supply plant tarnished profusely and caused such serious discoloration of the milk that it was found necessary to tin plate the cooler, in order to prevent recurrence.

On the other hand, Grindrod (6) who worked with sweet milk, found that "evaporated milk made in all-nickel or all-tin apparatus was very far superior to the ordinary evaporated milk" (meaning that made in copper vacuum pans). Again, an all-nickel horizontal coil vat installed in one of the factories of the Blue Valley Creamery Company (13) was used for the pasteurization and ripening of cream. This vat has proved entirely satisfactory as far as its effect on the flavor of cream and butter is concerned. It has been in daily use for a period of over two years and at no time has there been any indication of metallic flavor in the milk product, although the nickel has lost its original luster, shows considerable tarnish, and analysis of the cream by Quam (14) disclosed the presence of appreciable quantities of dissolved nickel. Hunziker and Hosman (9) found nickel to be equally satisfactory as tin in protecting butter against progressive flavor deterioration. Prolonged contact of nickel with butter failed to produce metallic flavor in the butter.

As may be seen from the above references, experiences with all-nickel equipment in milk plant operation under widely varying conditions are somewhat at variance. The present experiment deals with the behavior of nickel in milk work and may, therefore, help to explain some of the varied experiences found in its actual use. Nickel showed considerable solubility in the milk acids and it tarnished easily in most of the milk products, as well as in the individual weak organic acid solutions. In a few instances only a slight metallic flavor was detected in the milk product (acidophilus milk and sweet cream). It is evident, therefore, that nickel cannot be considered immune from the standpoint of metallic action in milk products and yet, under average conditions, it may well be looked upon as a reasonably safe metal for milk work. Its ready tarnishing and its tendency to slightly affect the flavor in the case of high-acid milk products upon prolonged exposure, constitute its chief drawbacks. As will be shown under Combination of Metals, the tarnishing of the nickel can be practically avoided by the presence of aluminum fittings in all-nickel equipment. Nevertheless, since nickel is fairly soluble it appears pertinent, here as in the case of tin, to question the desirability of the presence of considerable amounts of such metallic salts in food products.

Aluminum. Our results with aluminum suggest that this metal deserves favorable consideration in connection with milk plant equipment. With the exception of its instability in alkalies, as will be reported in a subsequent paper, its resistance to corrosion proved superior to tin and nickel products. In organic acids, sweet milk and cream, and moderately sour milk products, such as sour cream, buttermilk, and butter culture, corrosion and loss of weight were either entirely absent or only very slight. In dairy products of intensely high acidity, however, the corrosion was slightly more pronounced and a very slight metallic flavor was discernible. This was noticeable in high-acid milk, such as acidophilus milk.

The visible corrosion, tables 2, 5, and 7, was very slight. In most cases it was confined to a slight tarnish. This was noted chiefly in a few cases of high-acid milk and usually, but not always, in the presence of high temperature. The weight losses in the weak organic acids, table 1, while somewhat higher than those of the chromium steels, were far below those of any of the other metals. In the milk products, table 4, and in the neutralized cream experiment, table 7, the weight losses averaged practically the same as those for Allegheny Metal. The tendency to produce metallic flavor was no greater than in the case of nickel. There were no outstanding differences between the three aluminum products, i.e., pure aluminum, manganese-aluminum alloy containing 1.25 per cent manganese, and the same alloy plated with aluminum. The weight loss for each in the organic acids, the milk products, and the neutralized cream, averaged practically the same for all three metals, and no consistent difference could be observed in the visible corrosion of any of these three aluminum products nor in their effect on the flavor of the milk products.

It has been reported that in European milk plants where aluminum equipment is extensively used, because of its cheapness, the aluminum suffered considerable pitting in the presence of milk heated to high temperatures. These reports are not supported by observations by Hunziker (15) who has just visited numerous market milk plants in England, using aluminum pasteurizers, coolers, and holding tanks. This equipment had been in operation for a considerable time and showed no visible pitting nor objectionable tarnish. In the present experiment likewise, the aluminum products showed high resistance to corrosion and gave no evidence of pitting in milk and cream, hot or cold.

Another important factor greatly influencing the resistance of aluminum to corrosion in milk equipment is the physical state and purity of the metal. Thus, wrought aluminum, which is usually of relatively high purity, has been found to withstand attack far more readily than cast aluminum, which generally contains greater quantities of impurities. Seligman (1) points out that while tanks, cans, etc., made of sheet aluminum may be found unchanged after many years of use, cast fittings attached to the same containers may give out, after a few years' use. The cause of the short life of the cast metal in all probability lies in the presence of impurities, thus providing the necessary elements for the formation of electrical couples and giving rise to galvanic action. For similar reasons, contact of pure aluminum with other metals intensifies solution and pitting of aluminum, and as Seligman (1) suggests, the seriousness of the corrosion in such cases is intensified by the fact that the corrosion and pitting are often highly localized. In our own experiments aluminum rivets in nickel, Allegheny Metal, Ascoloy, and Enduro became markedly pitted and were partly eaten away. The aluminum rivets in tin were not affected. The relative softness of aluminum is an additional objection, particularly when subjected to rough handling. This suggests the advisability of heavy construction, especially in the case of large equipment. This objection is greatly decreased in the case of manganese-aluminum alloy which is a product of somewhat greater hardness as shown by Faragher (30).

These several drawbacks, while serious, may be readily minimized, if not entirely eliminated, by intelligent study of the properties of this metal and by suitable construction and proper use of the equipment. The cheapness, lightness, and comparative inertness in contact with the milk product recommend its recognition in milk work in this country.

Chromium steels. As previously shown, the chromium steels studied in this experiment were of two types, namely, ordinary chromium steels, or straight-chromium irons, so-called stainless steels, such as Ascoloy and Enduro; and the special chromium-nickel steel Allegheny Metal. These two classes of chromium steels differ from one another both chemically and physically. The ordinary chromium steels contain only a trace of nickel and are martensitic in structure. They are air-hardening above their critical temperature and decidedly magnetic. Allegheny Metal, the chromium-nickel steel, on the other hand, contains from 7 to 10 per cent nickel in addition to 17 to 20 per cent chromium. It is fully austenitic in structure, being non-hardening at any temperature and in addition, non-magnetic. Since our results with Allegheny Metal have already been given, the discussion here will be confined to the ordinary chromium steels, Ascoloy and Enduro.

Ascoloy and Enduro closely followed or were equal to Allegheny Metal in most cases, as far as visible corrosion and loss in weight are concerned. This was true for sweet and sour milks and creams, also for neutralized cream and for most of the organic acids. In hydrochloric acid and sulphuric acid both Ascoloy and Enduro corroded very profusely, lost greatly in weight, and dropped to the bottom of the list, together with zinc and iron. This suggests that when these ordinary chromium steels begin to yield to corrosion they may become very intensely affected, corroding and pitting rapidly. As will be shown in a later article, these ordinary chromium steels also suffered some corrosion under brine treatment. Furthermore, in high-acid milk, such as acidophilus milk, both Ascoloy and Enduro produced a slight metallic flavor. Whenever these two alloys suffered corrosion the Enduro proved slightly more resistant than the Ascoloy. This was probably due to the higher chromium content of Enduro, the latter containing approximately 16.5 to 18.5 per cent chromium and Ascoloy approximately 12 to 16 per cent. It was also noted in the case of Enduro, that the highly polished surface resisted corrosion considerably better than the unpolished back side. Similar findings are also reported by Quam (16), who found Allegheny Metal far superior to the ordinary chromium steels, and Burrell (29) who stated that "our experience with Uloy Enduro and other alloys containing Chromium, 13 to 16 per cent, but no nickel, was not satisfactory excepting in certain cases, that is, where the metal was thoroughly cleaned each day."

If one considers only the results obtained with the dairy products in this experiment, the ordinary chromium steels proved equally resistant to corrosion as Allegheny Metal, with the possible exception of metallic flavor in the high-acid acidophilus milk. However, if cognizance is taken of the extraordinarily heavy losses suffered in the weak mineral acids as shown in table 1, and the rusting in brine treatment as will be reported later, and also the metallic flavor observed in acidophilus milk, it appears that these ordinary chromium steels cannot be considered sufficiently dependable for all around use in dairy equipment on the basis of our present rather limited knowledge.

Copper, nickel silver, and Monel Metal. The metals of this group are similar in that they all contain copper which has proved undesirable for surfaces exposed to milk products.

As might be expected, copper showed itself the most objectionable of these three metals. In every milk product, tables 6 and 7, copper invariably yielded a pronounced metallic or related off-flavor. The effect on flavor here is augmented by the fact that the salts of copper themselves have an intense, puckery, metallic, bitter taste, which follows them in the dairy product. In fact, as Donauer (17) demonstrated, the intensity of metallic flavor of copper lactates is so great that less copper is required in solution to give the dairy product a metallic taste than is the case with any other metal he studied. Some of the metals with which Donauer experimented and the parts per million required to give a metallic taste are copper 4, nickel silver 5, aluminum 9, Monel Metal 10, iron 15, zinc 17, tin 50.

The visible corrosion on the copper strips, both in the weak organic acid solutions and in all the milk products, tables 2 and 5, was very pronounced and the loss in weight, tables 1, 4, and 7, comparatively high. Even in sweet milk and sweet cream the submerged part of the strips invariably became very bright, indicating corrosion and solution, and the portion above the liquid acquired a dark tarnish.

Similar brightening and tarnishing of copper in such dairy equipment as copper vacuum pans, hot wells, imperfectly tinned copper milk coolers and vats are common occurrences and often lead to serious flavor defects in the products of plants using such equipment. Thus Supplee (18) shows that milk heated or held in contact with metallic copper takes up the metal in amounts varying from less than 1 part per million to as high as 7.4 parts per million parts of milk. Supplee and Bellis (19) reported that the copper content of milk may be measureably increased by heating or storing milk in the presence of copper, or running the milk through poorly tinned copper pipes. Rice and Miscall (20) found that copper corroded with an oxide yields much more of the metal to the milk than smooth, bright copper. Hunziker (21) states that condensed milk often takes on a metallic flavor because of its contamination with copper from the vacuum pans. Recent work by Mattick (22) showed that an oily flavor in milk was very common in England and Scotland and he attributed this defect to extremely minute quantities of copper absorbed into the milk during cooling of the milk by the use of imperfectly tinned copper coolers. Grindrod (6) reports that the sterilization of milk and evaporated milk in an apparatus made of copper causes so great damage to the milk that his process of impact sterilization is not successful with an apparatus constructed of that metal.

The damaging effect on the milk product is further augmented by the fact that the presence in it of even very minute amounts of copper salts is capable of producing most intense progressive flavor deterioration in the dairy product with age, due to oxidation and catalytic changes, as shown by earlier work of Rogers, et al. (23) and Hunziker (24) in connection with the keeping quality of butter and by Supplee (18) as related to the keeping quality of milk powder.

Of the two copper alloys, nickel silver and Monel Metal, the former proved considerably more damaging to the milk product than the Monel Metal. Thus, nickel silver produced a metallic flavor in six of the milk products, while Monel Metal yielded this defect in only two cases. As shown in tables 6 and 7, with nickel silver the metallic flavor appeared in sweet milk, sweet and sour cream heated to 145°F., acidophilus milk, butter culture, and in one batch of neutralized cream. Likewise, the visible corrosion on the metal strips held in the milk products was much greater and the loss in weight larger for nickel silver than for Monel Metal. Both alloys suffered objectionable tarnishing in almost every case, but here again the action was decidedly more pronounced with the nickel silver than with Monel Metal. It was especially noticeable that this tarnish assumed a brass-like, reddish yellow color, suggesting oxidation of the copper rather than of the nickel. It appears that when the copper alloys yield to attack it is usually chiefly the copper that goes into solution. Thus the Research Laboratories of the Sheffield Farms (25) report that "milk treated with a piece of German silver (copper-zinc-nickel alloy) at 140°F. for six hours showed the presence of copper with traces of zinc and nickel." Seligman (1) found that "when attack does take place (meaning attack of German silver or nickel silver) it is mainly copper which passes into solution." These observations bear out our findings which showed that copper had passed into solution in the milk product exposed to nickel silver and Monel Metal, respectively.

In considering the suitability of these copper alloys for dairy equipment it should be emphasized that these alloys gave evidence of considerably greater resistance to corrosion than copper. This is especially true of Monel Metal and to a lesser extent of nickel silver. Their immediate damaging effect on the flavor of the dairy product also was distinctly less pronounced but appeared in several instances. Nickel silver caused metallic flavor more often than Monel Metal. On the basis of these findings the conclusion is inevitable that neither of these alloys gives dependable protection against metallic injury to the flavor of milk products. Their use should be confined to similar limitations as copper. These deductions are fully supported by commercial experience which, as shown by Burrell, Cornell, Hunziker, Hunziker and Hosman, and others, who demonstrated that these copper alloys in milk pails, vats, coolers, and similar equipment do, under certain conditions, cause serious flavor defects in the dairy product and can, therefore, not be recommended for use in milk work.

Aside from the damaging effect of copper salts on flavor and keeping quality of the milk product, the presence of such salts has been shown to be a menace to health. It is, therefore, pertinent to point out that the use of vacuum pans, hot wells, forewarmers, holding vats, pasteurizers, and similar equipment constructed in part or wholly of copper, or copper alloys, is highly undesirable, and yet much of the equipment used in present-day milk products plants daily exposes the product to these metal surfaces.

Iron, galvanized iron, tinned iron, and zinc. Iron proved itself most disastrous to the flavor of the milk products of any of the metals studied. As shown in tables 6 and 7 iron produced a most pronounced metallic flavor in all milk products and in many instances it appeared to cause intense putrefactive decomposition accompanied by foul flavor and odor. Similar results are also reported by Quam (26) who found that iron caused greater decomposition than any other metal. Likewise, the visible corrosion, tables 2 and 5, was most severe, both in the weak organic acids and in all the milk products; and the loss of weight, tables 1, 4, and 7, exceeded that of all other metals. In some cases the portion in the liquid was corroded so greatly as to make the iron appear silver white in color. In practically all cases the iron rusted severely above the liquid. The behavior of iron was so uniformly and highly damaging as to condemn its exposure to the milk product under any and all conditions, including black iron milk pipes, black iron steam pipes used for heating milk in the hot wells with direct steam, untinned iron milk pumps, rusty tinned iron equipment, and rusty milk and cream cans, etc.

The coating of iron with zinc failed utterly to furnish dependable protection. The intense corrosive properties of the zinc itself forfeit the resistance expected of the galvanized iron. While zinc produced metallic flavor in the sour milks and creams and in sour neutralized cream only, the galvanized iron caused metallic flavor in all milk products. In the case of both zinc and galvanized iron, the metallic flavor was sometimes accompanied by a sweetish taste and a marked reduction in acidity. Thus, in acidophilus milk, for instance, the acidity reached only 1.13 per cent whereas with the other metals it rose to about 1.76 per cent. In the case of galvanized iron, some of the milk products developed a fishy and bitter flavor as well.

The visible corrosion was very marked on both the zinc and the galvanized iron; the latter also showed marked rusting. The loss of weight of zinc in the dilute organic acids and the milk products averaged only about one-third of that of the iron, while the galvanized iron lost much more than did iron in the weak organic acids and equally as much as iron in the milk products. These findings are supported also by the work of Von K. Vas (27) who experimented with galvanized iron in sweet and sour whey and found that when exposed for only a few hours in the sweet whey the amount of zinc dissolved was very small, but in sour whey it amounted to 0.16 to 0.2 mgm. per square centimeter, which he considers condemns galvanized iron for use in the construction of whey tanks from the health standpoint.

It is evident from the above results that galvanized iron is exceedingly destructive to the flavor of milk products. This, together with the known toxic properties of zinc salts when present in large quantities, condemns the use of galvanized iron in any form of dairy equipment, such as milking pails, milk and cream pipes, buttermilk and whey tanks, etc.

The tinned iron proved far superior to either galvanized iron or iron. Yet in the majority of the milk products it produced a pronounced metallic flavor. The visible corrosion was considerable and, on many of the strips iron rust appeared through the tin. The loss in weight also averaged second only to iron, galvanized iron, and zinc, but in many cases exceeded that of even copper. While a heavy and uniform coating of tin does give satisfactory protection against corrosion of the iron, such perfection of tin coating on iron is very rare. Moreover, the tin coating is porous, inviting access of the media to the easily corroded iron underneath. This provides corrosion centers on which action is concentrated and from which corrosion spreads rapidly. The damage is further aggravated by the use of strong caustic alkalies for washing purposes, which readily attack the tin, as will be shown in a succeeding article. As a convincing example of this fact may be cited commercial experience in the washing of farmers' milk and cream cans with solutions of caustic alkalies. Such cans lose their tin coating rapidly, rust profusely, and soon are in a bad and insanitary condition. It is well known, though not fully appreciated, that such cans are responsible for a great deal of the metallic flavor in cream that arrives in American creameries. There is no question that the factor of rusty cream cans plays a very important rôle in the production of cream of poor quality.

Combinations of metals. The presence of two or more metals in the same piece of dairy equipment is fundamentally undesirable. Most metals used may differ in their electrical potentials and this in turn invites corrosion by electrolysis. For similar reasons, impurities in metals are equally objectionable, as was pointed out in the case of cast aluminum. The corrosion-inciting effect of contact with other metals in the case of aluminum and chromium steel equipment, has already been explained under the respective headings of these metals.

Certain alloys have proved unsuitable for milk work because they, too, provide the elements for electrical couples. This appears to be especially the case with copper alloys, such as nickel silver, and to a lesser extent Monel Metal. When these alloys corrode the metal that goes in solution is usually found to be the copper. It is well known by the metallurgist that in the case of nickel silver, for instance, segregation may take place during the cooling of the molten alloy, thus resulting in the formation of electrical couples which, when submerged in the milk product, may incite electrolysis. The same situation prevails in equipment consisting of metal-coated surfaces, such as tinned iron, galvanized iron, tinned copper, whenever these coatings become defective, and cause exposure of the metal underneath the coating.

Our experiments on the effect of combination of metals consisted of using strips of Allegheny Metal, Ascoloy, Enduro, nickel, and tin, which had fastened through the center of the strips rivets of the following metals: brass, copper, iron, Monel Metal, nickel, nickel silver, tin, aluminum, and solder. These strips were then totally immersed in various media, such as lactic acid, sour cream, sweet milk, etc.

The aluminum rivets showed marked corrosion and pitting in the acid products, such as in lactic acid and sour cream, except in case of the tin strip in which neither rivet nor strip was affected. In the case of the nickel strip the nickel appeared not to be affected while it tarnished in all other instances, but the aluminum rivet was much corroded. The two ordinary chromium steels were quite corroded over a considerable area around the aluminum rivet. Ascoloy was corroded the most and Enduro next. Allegheny Metal was unaffected by this aluminum rivet combination.

The iron rivets were badly rusted in all cases except the rivet in the tin strip. In the case of lactic acid all three chromium steels were corroded and markedly tarnished but the Allegheny Metal was least affected.

The tin rivets also caused the three chromium steels to tarnish and corrode severely, less so, however, than did the iron rivets. The Allegheny Metal here, too, was least affected. All other combinations of metals and rivets gave results practically identical to those obtained with the metal strips alone.

It appears from the above, therefore, that combinations of the chromium steels with aluminum, tin, or iron may prove quite disastrous to the chromium steels, causing them to tarnish and corrode markedly. Aluminum appears to have an inhibiting effect on the tarnishing of nickel but the aluminum itself is considerably affected, while in the aluminum-tin combination neither metal showed attack.

The complications involved in these tests with combinations of metals so greatly limited the scope of this part of the experiment that the results are inadequate to draw more comprehensive conclusions. However, they may serve to show the importance of avoiding multiplicity of such metals in one and the same piece of equipment, of confining the construction of mechanical accessories and fittings that come in contact with the milk, as completely as possible to the metal of which the milk side of the equipment consists, or if different metals must be used, of giving preference to metals of similar electrical potentials.

While, for above reasons, the presence of more than one metal immersed in the milk product is undesirable, there are instances, as noted above, when the introduction of a second metal may not only be harmless, but may actually be beneficial in that it may assist in retarding corrosion of the metal it is desired to protect. Thus Quam (16) also points out that aluminum, manganese, zinc, and wrought iron materially retard the corrosion of nickel and tin. Burrell (5) and McKay (28) likewise report that aluminum noticeably prevents the discoloration and pitting of nickel. In these cases galvanic action is utilized to protect the other metal against attack.

CORROSION AT THE AIR-LIQUID LINE

It is well recognized by students of the subject of metallic corrosion, that the amount of dissolved oxygen present in a solution influences to a very marked degree the extent and type of corrosion that any given metal will suffer when immersed in the liquid. In the present experiment the air-liquid line furnished convincing evidence of the fact that often the most intense action will take place at points where the liquid is most freely exposed to the air. This was true both in the weak organic acid solutions and in the milk products. In some instances the corrosion was so great as to result in a deep cut at the air-liquid line. In the weak organic acid solutions the corrosion at the air-liquid line was most pronounced in the case of iron, galvanized iron, tinned iron, zinc, copper, nickel silver, and Monel Metal. It was less intense but distinctly noticeable on nickel, tin, tinned copper, and the aluminum products, very slight on the ordinary chromium steels, Ascoloy and Enduro, and entirely absent on

Allegheny Metal. In the milk products the corrosion at the air-liquid line was greatest in the case of iron, galvanized iron, zinc, copper, and nickel silver, less pronounced but still noticeable in many instances on nickel, Monel Metal, tinned iron, and tinned copper, and practically completely absent on tin, the aluminum products, and the chromium steels.

In acid liquids and at the higher temperatures the action near the surface of the liquid was usually greater than in the sweet products and at ordinary temperatures, although marked corrosion at the air-liquid line of some of the metals was also noticeable in the sweet milk and cream at ordinary temperatures.

These findings are in harmony with experience in commercial plant operation which involves many instances where exposure of the wet metal surface in dairy equipment to air occurs and where at such points of contact corrosion and tarnishing of the metal is more intense than where the metal is completely submerged. As striking examples here may be mentioned the blackening and spotting of tinned copper at the air-liquid line in coil cream vats and on the revolving coils themselves, the rapidity with which surface tube coolers show severe tarnishing, the rapid rusting of milk and cream shipping cans that are returned to the farms in wet condition, the ready tarnishing of copper forewarmers and vacuum pans, the intense corrosion of steel-jacketed tanks, where the jacket is emptied but remains wet after each day's operation. The action is usually further augmented in equipment exposed to direct sunlight.

Toxicity of metals. The present experiment did not include the toxicological aspect of metals. Furthermore, the scarcity of comparable and tangible data available renders a discussion of this subject difficult. There appears to be no established standard of toxicity on the basis of which the several metals herein considered may be compared with reasonable accuracy. Since it is generally conceded, however, that salts of such metals as chromium, copper, zinc, and tin, are highly poisonous, it appears expedient to emphasize here that the solubility of these metals in milk work may be a factor of considerable importance, and that the use in dairy equipment of such metals of which large amounts go into solution, may be considered a questionable practice. This would apply to their direct physiological effect on the human system as well as to their inactivating influence on the valuable milk vitamins.

SUMMARY AND CONCLUSIONS

This experiment involves a study of the various metals used in dairy equipment. The action on the metals of weak organic and mineral acids and of sweet and sour milks and creams, and the effect of the metals on the flavor of the milk products were observed.

The metals used included aluminum and aluminum alloys, copper, nickel and copper alloys, iron, zinc, and galvanized iron, tin, tinned copper and tinned iron, two ordinary chromium steels and one chromium-nickel steel. The liquids used consisted of 1 per cent solutions of the following acids: hydrochloric, sulphuric, acetic, butyric, and lactic, and 0.2 per cent solutions of citric; and of the following milk products: sweet milk, acidophilus milk, butter culture, and buttermilk, sweet and sour cream, and sour neutralized cream. The metal strips were immersed half way in the liquids and the periods and temperatures of exposure were five days at 70°F. and five hours at 145°F.

In general, the corrosive effect on the metal was greater in the acid solutions than in the milk products. This was evident even where the acidity of the milk product was equal to or greater than that of the acid solution. This suggests that the acidity is the chief factor in metallic corrosion and that some of the non-acid milk constituents apparently exert a corrosion-protective action.

With minor exceptions, the corrosion was more intense in the high-acid products than in the low acid products and at high temperatures than at room temperature. But even in the sweet milk products and at room temperature corrosion was by no means absent.

Generally those metals that showed definite corrosion also had the most damaging effect on the flavor of the milk product. This refers particularly to iron, galvanized iron, copper, zinc, tinned iron, and nickel silver, the first three of which produced a marked metallic flavor in all milk products and the last three in the majority of cases.

Allegheny Metal, tin, and properly tinned copper produced no damaging flavor effect whatever. These were closely followed by the aluminum products, nickel, Monel Metal, and the ordinary chromium steels. The latter group of metals, however, were not entirely negative and did produce a metallic flavor in some instances.

The accurate placement of these metals on the basis of the results here recorded according to their suitability for use in dairy equipment is difficult. Some of the metals, while harmless in dairy products of low or moderate acidity, showed a marked tendency to impair the flavor in the high-acid products. Others, while practically harmless in most of the dairy products showed lack of resistance to corrosion under certain severe conditions and, therefore, are not sufficiently dependable to be recommended for general use.

While the effect on the flavor of the milk product must ever be considered of paramount importance, a consistent classification of these metals must, in addition, take into consideration their dependability to stand up under a great variety of conditions, such as are encountered in commercial plant operation, which includes also resistance to steam, washing solutions, and cooling brines, etc. With these numerous aspects in mind the following alignment according to relative merit appears justifiable:

1. Allegheny Metal, tin, and heavily tinned copper.

   These metals have no effect on flavor and show maximum resistance to corrosion and tarnishing. The tin coating on copper must be heavy and intact.
2. Nickel, aluminum, and manganese aluminum alloy.

   These metals are not entirely satisfactory in high acid milk products. Furthermore, the nickel tarnishes readily and the aluminum is sensitive to alkali washing powders.
3. Monel Metal, Enduro, Ascoloy, and nickel silver.

   Monel Metal tarnishes appreciably in milk products. In these tests it had only slight effect on flavor. Enduro and Ascoloy are sensitive to high-acid products and their performance in dairy equipment is uncertain. Nickel silver tarnishes severely and was injurious to flavor in the majority of milk products.

4. Tinned iron, copper, galvanized iron, iron, and zinc.

With the exception of properly tinned iron, this group is unfit for use in contact with milk products. Tinned iron is equally so whenever iron is exposed.

ACKNOWLEDGMENTS

We are greatly indebted to the Allegheny Steel Company, the Aluminum Company of America, D. H. Burrell & Company, J. G. Cherry Company, The Creamery Package Manufacturing Company, The International Nickel Company, and the Jensen Creamery Machinery Company, for materials and data generously placed at our disposition.

REFERENCES

(1) Seligman, R. 1923 Milk and metals. Proc. World's Dairy Congress, ii, 1202.

(2) Hunziker, O. F. 1923 Selection of metals in the construction of dairy equipment. Proc. World's Dairy Congress, ii, 1189.

(3) North, C. E. 1927 Report on chemical and electrical tests on metals. For The Creamery Package Manufacturing Company.

(4) Quam, G. N. 1927 Conduct of stainless steel in sweet and sour milk. Report by J. G. Cherry Company.

(5) Burrell, L. 1927 Information by correspondence.

(6) Grindrod, Geo. 1927 Information by correspondence.

(7) Dahle, C. D. 1927 Information by correspondence.

(8) MacQuigg, C. E. 1926 Chromium alloys in chemical plant equipment. Chem. and Met. Eng., xxxiii, 609.

(9) Hunziker, O. F., and Hosman, D. Fay 1917 Tallowy butter, its causes and prevention. Jour. Dairy Sci., i, 77–106.

(10) Mantell, C. L., and Lincoln, E. S. 1926 Corrosion of tin plate in containers. Chem. and Met. Eng., xxxiii, 622.

(11) Merica, P. D. 1917 Structure of the coating on tinned copper in relation to a specific case of corrosion. U. S. Bur. of Stand., Bull. No. 90.

(12) Cornell, F. G., Jr. 1927 Information by correspondence with the Jensen Creamery Machinery Company.

(13) Blue Valley Creamery Co. 1927 Investigation in the use of all-nickel equipment.

(14) Quam, G. N. 1926 Solubility of nickel in milk. Report by J. G. Cherry Company.

(15) Hunziker, O. F. 1928 Report to U. S. Dept. of Agr. on dairy equipment exhibition at Royal Agri. Show, Nottingham, England.

(16) QUAM, G. N. 1926 Effect of milk on metals. Report by J. G. Cherry Company.

(17) DONAUER, MAX 1922 The action of metals on milk. The Ice Cream Rev. Feb. issue.

(18) SUPPLEE, G. C. 1923 The keeping quality of dry milk. Proc. World's Dairy Congress, ii, 1248.

(19) SUPPLEE, G. C., AND BELLIS, B. 1922 The copper content of cow's milk. Jour. Dairy Sci., v, 455.

(20) RICE, F. E., AND MISCALL, J. 1923 Copper in dairy products and its solution in milk under various conditions. Jour. Dairy Sci., vi, 261.

(21) HUNZIKER, O. F. 1926 Condensed Milk and Milk Powder, 4th ed., p. 398.

(22) MATTICK, A. T. R. 1927 Oiliness in milk. Jour. Agr. Sci., xvii, 388.

(23) ROGERS, L. A., BERG, W. N., POTTEIGER, C. R., AND DAVIS, B. J. 1913 Factors influencing the change in flavor of storage butter. U. S. Dept. Agr., B. A. I. Bull. 162.

(24) HUNZIKER, O. F. 1927 The Butter Industry, 2nd ed., pp. 49 and 503.

(25) Sheffield Farms' Research Laboratories 1915 Investigation of cause of metallic flavor in milk at Pawling, N. Y. Plant.

(26) QUAM, G. N. 1926 The effect of metals on the putrefactive decomposition of milk. Report by J. G. Cherry Company.

(27) VAS, VON K. Verhalten von Zink- und Kupferplatten in Milch und Molke. Kisérletügy Kozlemények, xxvi, 7.

(28) McKAY, R. J. 1927 Information by correspondence with International Nickel Company.

(29) BURRELL, L. 1928 Information by correspondence.

(30) FARAGHER, P V. 1927 Commercial forms and applications of aluminum and aluminum alloys. Tech. Publ. No. 33, Amer. Inst. Min. and Met. Eng.

THE BACTERIAL COUNT OF ICE CREAM HELD AT FREEZING TEMPERATURES*

JOHN WEINZIRL AND ALICE E. GERDEMAN

Department of Bacteriology, University of Washington, Seattle

During the course of an earlier investigation on ice cream (1), it was observed that the total counts of bacteria in samples received from retailers were much higher than in those received from producers. It is generally assumed that a freezing temperature inhibits the growth of bacteria. If this assumption is true, then the increased counts found in the retailers' samples would seem to be due to insanitary methods. However, the increase in numbers was of such a magnitude as to render this simple explanation questionable. It occurred to us that it might be helpful to investigate this problem and to determine, if possible, the cause of the increase noted. In this investigation we determined the effect of low temperatures upon the bacterial count of ice cream.

REVIEW OF LITERATURE

A review of the available literature casts considerable doubt upon the ability of freezing temperatures to completely inhibit bacterial multiplication in ice cream. Fabian (2) states that in most cases when ice cream is held at low temperatures, there is usually a slight decrease for two to four days, then an increase for ten days, after which there is a gradual decrease, and finally a fall below the original count. Stiles and Pennington (3) made counts of four samples over a period of thirty-four days. The counts increased slightly to the fourth day, then decreased to the fourteenth day when a marked increase occurred followed by a sharp decline after the twenty-seventh day. Similar findings were reported by Ellenberger (4).

---

* Received for publication October 24, 1928.

On the other hand, Tracy and Peterson (5) kept ice cream mix at 32° to 35°F. for a period of thirty-two days, but found no marked increase in numbers of bacteria until after the fourteenth day. Hammer (6) did not find any tendency of the bacteria to increase in numbers during the storage of ice cream. Esten and Mason (7) studied twelve samples of ice cream over a period of two months, but found no consistent increase or decrease. Hammer and Goss (8) examined 51 samples of ice cream of which 39 were packed in salt and ice, and 12 were stored in the hardening room. They found that during proper storage there is no increase and there may be a decrease in the number of bacteria present. Fay (9), Palmer (10), and Rettger (11) found that if ice cream was held at a suitable temperature, there was a material drop from time to time in the viable bacteria present. According to Fabian and Cromley (12) storing the product at −20.5°C. for ten to twenty-one days decreases the plate count in the majority of cases, but freezing with brine at an average temperature of −14°C. permits bacterial multiplication.

The varying temperatures employed by the different workers leaves some doubt as to what actually occurs during cold storage, but in a general way, they seem to indicate that storing at temperatures above −14°C. did not completely inhibit bacterial multiplication.

METHODS EMPLOYED

Source of samples. The ice cream came from two wholesale makers and it was delivered to the laboratory soon after freezing. The samples were then placed at the desired temperature for thirty days, bacterial counts being made every other day.

Method of analyzing. Portions of the ice cream were removed to sterile petri dishes, placed in the incubator at 37°C. for ten minutes to liquefy, and then serial dilutions up to 1:1,000,000 were made. One cubic centimeter portions were plated with standard beef extract agar. Triplicate plates were made from each dilution, incubated for forty-eight hours at 37°C. and the colonies counted. Only the plates showing between 50 and 300 colonies were counted and averaged for the final figures.

Method of storage. The ice cream was stored in the freezing compartment of a Frigidaire cooler, the temperature of which was carefully checked. Twelve samples were stored at −3°C., 12 at −6°C., and 9 at −10°C. The temperatures remained constant during the experiment.

RESULTS OBTAINED

At −3°C. The samples were purchased in lots of four each from a single maker "X." Table 1 gives the results for each sample of the first lot. Since the four samples came from a single batch of ice cream, they do not differ greatly.

TABLE 1

Giving bacterial counts (in millions per gram) of four samples of ice cream held at −3°C. for thirty days

DAYS STORED	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
1	26.1	31.0	29.0	28.9
3	31.2	30.0	29.8	34.0
5	32.0	35.0	32.0	40.0
7	74.0	57.0	58.0	81.0
9	121.0	118.0	108.0	133.0
11	137.0	132.0	129.0	151.0
13	149.0	144.0	151.0	163.0
15	191.0	197.0	241.0	257.0
17	184.0	176.0	192.0	201.0
19	161.0	158.0	174 0	169.0
21	122.0	133.0	145 0	139.0
23	99.0	104.0	112.0	121.0
25	76.0	89.0	93.0	89.0
27	65.0	67.0	63.0	76.0
29	44.0	52.0	45.0	51.0

The table shows that the four samples of ice cream had approximately the same number of bacteria at the same time during the experiment, and the number remained practically constant during the first five days of storage, then increased rapidly to the fifteenth day, when a more gradual decline set in.

Since the subsequent lots were handled like the first it scarcely seems worth while to give all the detailed figures as determined;

instead, only the average of the lot of four samples will be given. Table 2 gives the averages of all the lots held at −3°C.

The averages all show the same trend as do the results of the specific samples, namely, the first five days at −3°C. shows a comparative quiescence with only a slight increase, then a fairly rapid increase for ten days, followed by a decline which was quite rapid at first, then proceeded more slowly.

At −6°C. The samples stored at −6°C. were 12 in number, all from a single producer, "Y." They were divided in two lots of six samples each. The samples had a lower initial count than those from "X." Again the counts for all the samples in each lot are averaged and the averages given in table 3.

TABLE 2

Giving averaged bacterial counts (in millions per gram) for each lot of four samples held at −3°C.

DAYS STORED	LOT I (SAMPLES 1, 2, 3, 4)	LOT II (SAMPLES 5, 6, 7, 8)	LOT III (SAMPLES 9, 10, 11, 12)
1	29.0	36.3	37.0
3	31.0	37.5	39.3
5	34.8	50.5	42.8
7	70.0	70.8	74.3
9	120.0	117.8	127.8
11	137.3	142.0	169.5
13	151.5	176.8	198.8
15	221 5	203 3	224.5
17	188.3	181.8	229 5
19	165.5	148.3	181.0
21	134.8	127.0	152 0
23	109.0	107.8	134 3
25	86.8	85 8	120.0
27	67.8	68.3	100.5
29	48 0	57.3	84 0

As may be seen the results at −6°C. parallel those at −3°C. in a general way. The initial number of bacteria was very much smaller than in the samples from "X," and these numbers always remained relatively small, but the maximum occurred again about the fifteenth day. The counts at the end of the period of storage were lower, however, than the initial counts, tending to show increased inhibition due to increased cold.

TABLE 3

Giving averaged bacterial counts (in millions per gram) for each lot of six samples of ice cream held at −6°C. for 30 days

DAYS STORED	LOT IV (SAMPLES 13, 14, 15, 16, 17, 18)	LOT V (SAMPLES 19, 20, 21, 22, 23, 24)
1	0.7	1.3
3	0.8	1.4
5	1.0	1.3
7	1.1	1.3
9	1.3	1.4
11	1.5	1.6
13	1.6	1.7
15	1.8	2.1
17	1.5	1.9
19	1.3	1.7
21	1.1	1.6
23	0.9	1.3
25	0.8	1.0
27	0.5	0.9
29	0.5	0.8

TABLE 4

Giving averaged bacterial counts (in millions per gram) of two lots of ice cream held at − 10°C. for thirty days

DAYS STORED	LOT VI (SAMPLES 25, 26, 27, 28, 29)	LOT VII (SAMPLES 30, 31, 32, 33)
1	0.9	1.5
3	0.7	1.0
5	0.4	0.6
7	0.9	0.7
9	1.0	0.8
11	1.2	1.0
13	1.7	1.3
15	1.8	1.6
17	2.0	1.9
19	1.8	2.3
21	1.5	2.1
23	1.1	1.9
25	1.0	1.8
27	0.9	1.6
29	0.8	1.4

At −10°C. All the samples stored at −10°C. came from producer "Y." They were divided into two lots of 5, and 4 samples each. The averages for each lot are given in table 4.

The results at −10°C. differ from those at −3°C. and −6°C. in that there occurred an initial decrease. This decrease was followed after a number of days by an increase as in the previous samples. This increase was followed by a gradual decline.

DISCUSSION OF RESULTS

Viewing the results as a whole they seem definitely to indicate that temperatures between zero and −10°C. do not inhibit the multiplication of some of the species of bacteria found in ice cream. These findings parallel quite closely the results obtained by Fabian (2), and in a general way those of Stiles and Pennington (3), and of Ellenberger (4). They tend to show that progressive lowering of the temperature decreases the amount of multiplication and they point quite definitely to some temperature at which multiplication must cease. Doubtless some of the workers reached such low temperatures; if they did, our findings do not necessarily disagree with theirs.

The variation in results obtained by others might depend upon a variation in the bacterial flora. Our samples came from only two producers, "X" and "Y," hence may not truly represent all the possible floras. We made only a partial study of the psychrofilic flora present, and found the species belonged largely to the non-sporebearing types present in water and soil. Conceivably, if these were killed by pasteurization, there might be no multiplication of the species remaining.

We also found, as did Hammer (6), that bacteria forming gas in lactose bouillon tend to disappear during cold storage and frequently died out completely in ice cream stored for thirty days.

Our results furnish an explanation of the original question that confronted us, namely, the greatly increased numbers of bacteria found in retailers' samples of ice cream as compared with those from the makers. Obviously the refrigeration employed by retailers permits the multiplication of some of the bacteria present. Hence, it is not necessary to invoke unsanitary methods of handling to account for the numbers of bacteria, although this may still be a factor in some cases. The results are also important in the consideration of bacterial standards for ice cream. It would seem that any standard adopted should make allowance for the increase in numbers due to holding the product. The California Standard limiting the number of bacteria per gram to 150,000 would seem to work an undue hardship on many of the retailers, if the results of Weinzirl and Harris (1) are representative of conditions in general.

SUMMARY AND CONCLUSIONS

1. Thirty-three samples or bricks of ice cream from two producers were kept at temperatures below zero centrigrade and examined for numbers of bacteria per gram on alternate days. Twelve samples were held at −3°C., 12 at −6°C., and 9 at −10°C.
2. The results indicated that storage of ice cream at −10°C. or above does not prevent all bacterial multiplication or increase in the bacterial count.
3. The results furnish at least a partial explanation of the increased numbers of bacteria found in retailers' samples as compared with those from producers.

REFERENCES

(1) Weinzirl, John, and Harris, L. S.: A comparison of certain methods for determining the sanitary quality of ice cream. Jour. Dairy Sci., 1928, xi, 284–291.

(2) Fabian, F. W.: Production of ice cream with low bacterial content. Mich. Agric. Exp. Sta., Spec. Bul. 159, 1927, 1–12.

(3) Stiles, G. W., and Pennington, M. E.: Bacteriological investigation of ice cream in the District of Columbia. Hygienic Laboratory, Bul. 41, 1908, 249–263.

(4) Ellenberger, H. B.: A study of bacteria in ice cream. Cornell Agri. Exp. Sta., Memoir 18, 1919, 331–362.

(5) Tracy, P. H., and Peterson, R. W.: The condensation process of preparing an ice cream mix. Jour. Dairy Sci., 1922, v, 273–281.

(6) Hammer, B. W.: Bacteria and ice cream. Iowa Agric. Exp. Sta., Bul. 134, 1912, 279–301.

(7) Esten, W. M., and Mason, C. J.: Bacterial content of ice cream. Storrs Exp. Sta., Bul. 83, 1915, 128–134.

(8) Hammer, B. W., and Goss, E. F.: Bacteria in ice cream. Iowa Agric. Exp. Sta., Bul. 174, 1917, 1–21.

(9) Fay, A. C.: Bacterial content of some Kansas ice creams. Jour. Dairy Sci., 1923, vi, 283–291.

(10) Palmer, H. G.: Bacteriological problems in ice cream manufacture. Creamery and Milk Monthly, 1922, xi, 69–70.

(11) Rettger, Leo F.: Influence of carbon dioxide on bacteria. Rept. Nat'l Assn. of Ice Cream Manufacturers, 1922, 10–26.

(12) Fabian, F. W., and Cromley, R. H.: The influence of manufacturing operations on the bacterial content of ice cream. Mich. Agric. Exp. Sta., Tech. Bul. 60, 1923, 5–23.

ARTHUR CHRISTOPHER BAER

Arthur Christopher Baer

1884-1929

The passing of Arthur Christopher Baer at Stillwater, Oklahoma, on the seventh day of January, nineteen hundred twenty-nine, is a great loss to all who are interested in the dairy industry throughout the United States and the world.

Thirteen years ago, Professor Baer, then instructor at the University of Wisconsin, was asked to come to the Agricultural College of Oklahoma to assume head of the Dairy Department. During these past years he worked untiringly to advance the cause in which he was so much interested and in which he was so well equipped with knowledge and experience.

In his young manhood, Professor Baer studied under some of the outstanding men in the dairy industry; notably, Dr. Babcock, Dean Henry and Professor Farrington. In the later years of his life he was looked upon as an authority—particularly in the manufacture of ice cream—having written a number of publications on the subject. For several years his health had been failing but he was reluctant to take the rest urged by both friends and physicians. The last year he gave better than his strength in superintending and equipping a fine, new dairy building at the Oklahoma Agricultural College. It seems very sad that the things for which he dreamed and planned all these years should not have been completed before his untimely death.

A magnificant dairy barn and a new creamery building, both the results of his efforts, will now stand as monuments to his untiring interest in building up the industry of dairying for the state he served so faithfully and well. Not alone in these monuments of brick and stone will his memory be perpetuated but in the memory of thousands of students and associates who were fortunate enough to enjoy and profit by his teachings and writings.

Arthur Christopher Baer was born in West Bend, Wisconsin, February 4, 1884. He died in Stillwater, Oklahoma, January 7, 1929; aged 44 years, 11 months and 3 days. He leaves his widow and daughter, Virginia, aged 10 years, and three sisters and two brothers who reside in Wisconsin.

He received his education in the West Bend, Wisconsin public schools. His collegiate work was taken in the University of Wisconsin where he gained recognition as an honor student and obtained his Bachelor's degree in 1911. For a short time following graduation, he practised dairy farming, later becoming an instructor in the University of Wisconsin where he taught until 1916. In that year he resigned to accept a position as head of the Dairy Department of the Oklahoma Agricultural and Mechanical College, which position he held until his untimely death.

Professor Baer was active in public affairs. During the World War he rendered valuable service in the liberty bond drives. He was active in the local chapter of the American Red Cross and for sometime served as its chairman. He was a member of the Alpha Zeta, honorary agricultural fraternity; Alpha Gamma Rho, professional agricultural fraternity, and a 32-degree Mason.

He was active in the American Dairy Science Association, having served as chairman of the Dairy Manufactures Section and the Southern Division and as vice-president of the Association.

THE USE OF EGG YOLK IN ICE CREAM*

W. H. MARTIN AND W. J. CAULFIELD

Eggs have long been used in the making of home made and French ice cream, and recently the use of dehydrated eggs by many ice cream manufacturers has become a common practice. Whether or not the regular use of egg yolk in ice cream is justified is a debated question at the present time. Unfortunately the literature is lacking in information on this particular subject. Dahle (1), Turnbow and Rafetto (2), Sommer (3), and others have pointed out in a general way some of the benefits derived from the use of egg yolk, but for the most part experimental data are lacking.

The lack of information on this subject prompted the writers to conduct some investigations to determine what benefits might be expected from the use of egg yolk in commercial ice cream. The investigation dealt with the effect of egg yolk on the whipping qualities of mixes and its effect on the body, texture and flavor of the resulting ice cream.

EXPERIMENTAL METHODS

Commercial dehydrated egg yolk was used, a sufficient quantity being purchased so that the same lot of egg yolk powder was used throughout. The egg yolk was prepared by dissolving the powder in water. A total solids determination, using the method as outlined by Mojonnier and Troy (4), was made on this solution to determine the percentage of egg yolk solids present. The calculated amount of this egg yolk solution was weighed out on a gram scale and added to the mixes prior to pasteurization, with one exception to be noted later.

Fifty pound mixes were made from cream, plain condensed skim milk, skim milk, sugar, and gelatine, the correct quantity of each

---

* Received for publication November 23, 1928. Contribution No. 61 from the Department of Dairy Husbandry, Agricultural Experiment Station, Kansas State Agricultural College, Manhattan, Kansas.

ingredient being carefully calculated and weighed. The mixes were pasteurized at 145°F. for thirty minutes and homogenized at 2000 pounds pressure with a single stage Gaulin homogenizer. The mixes were cooled to approximately 40°F. by means of a surface cooler and aged at that temperature unless they were frozen immediately.

Freezings were made in a Cherry fifty quart brine freezer. In all cases the freezer was pre-cooled by freezing a preliminary batch prior to freezing any mixes for which data were recorded. Overrun determinations were made at minute intervals after the first two-minute period by means of a Mojonnier overrun tester which was adjusted for each group of mixes studied. The brine was shut off in all cases when the mixes reached the desired consistency for whipping. A pint sample of ice cream from each mix was obtained at the freezer at approximately the same overrun, placed in the hardening room and later examined for body, texture, and flavor.

EFFECT OF EGG YOLK ON THE WHIPPING QUALITIES OF HIGH SOLID MIXES AND ON THE TEXTURE OF THE ICE CREAM

A group of 7 mixes calculated to contain 12 per cent fat, 12 per cent serum solids, 15 per cent sugar, 0.4 per cent gelatine, and egg yolk varying from 0 to 0.4 per cent were made up, processed as previously described and frozen. Two of the 7 mixes, one containing no egg yolk and the other containing 0.4 per cent egg yolk, were frozen fresh, whereas the remaining five were frozen after aging for twenty-four hours. The data relative to the freezing of these particular mixes are expressed in table 1.

The results recorded in table 1 indicate that egg yolk is beneficial in shortening the time required to obtain the desired yield with a mix of this composition. The attention of the reader is especially directed to the data in table 1 relative to the two mixes which were frozen fresh. A saving of 8 minutes in freezing time will be noted in favor of the fresh mix containing 0.4 per cent egg yolk as compared with the same mix containing no egg yolk. The fresh mix containing no egg yolk whipped very slowly in the freezer and before the desired yield was obtained it was too soft to draw from the freezer. The duplicate of this mix, but containing 0.4 per cent egg yolk not only whipped faster but it maintained a relatively firm consistency throughout, so that it was in excellent condition for placing in the hardening room at the time it was drawn from the freezer.

TABLE 1

Effect of egg yolk on overrun and freezing time of fresh and aged mixes calculated to contain 12 per cent fat, 12 per cent serum solids, 15 per cent sugar, and 0.4 per cent gelatine

Brine temperature + 4°F.

FREEZING TIME	PER CENT OVERRUN						
	Fresh, no egg	Fresh, 0.4 per cent egg	Aged, no egg	Aged, 0.2 per cent egg	Aged, 0.3 per cent egg	Aged, 0.4 per cent egg	Aged, 0.4 per cent egg added after homogenization
minutes							
2	40	23	33	35	25	27	20
3	50	29	44	45	30	33	26
4	57	36	47	50	36	41	32
5	56	40	49	51	41	47	39
6	53	47	51	52	47	52	46
7	52	54	54	55	55	58	52
8	51	70	56	56	61	64	59
9	52	77	59	61	71	74	68
10	56	82	64	70	78	83	78
11	60	93	69	76	86	92	84
12	63	102	75	83	94	100	92
13	67		80	90	102		98
14	71		85	98			
15	76		90				
16	79		95				
17	82						
18	85						
19	89						
20	93						

The data expressed in table 1 indicate that the benefits from the use of egg yolk are more apparent when the mixes are frozen fresh. It will be noted that as the concentration of egg yolk increased the freezing and whipping time decreased. A saving of 4 minutes and a gain of 5 per cent in overrun was effected in the aged mixes through the use of 0.4 per cent egg yolk. It is logical to expect that egg yolk should improve the whipping qualities of relatively slow freezing mixes of this type.

To note the effect of adding egg yolk before and after homogenization 0.4 per cent egg yolk was added to one of the mixes after homogenization. The mix was then aged twenty-four hours and frozen. The data shown in table 1 indicates that it is important to homogenize the egg yolk with the mix if the maximum benefits are to be secured from its use.

An examination of the ice cream samples obtained at the freezer from each of these mixes at approximately 95 per cent overrun showed all samples to be smooth in texture whether egg yolk was present or not. The presence of egg yolk could not be detected with any degree of certainty from the flavor or appearance of these particular samples. In the case of high solid mixes of this type very little if any improvement will be noted in the body and texture of the product as a result of using egg yolk. On the other hand, a considerable saving in freezing time was effected through the use of egg yolk with this particular type of mix.

THE EFFECT OF EGG YOLK ON TEXTURE AND OVERRUN WHEN USED WITH MIXES OF LOW TOTAL SOLID CONTENT

Six individual mixes calculated to contain 12 per cent fat, 8 per cent serum solids, 15 per cent sugar, 0.4 per cent gelatin, and egg yolk varying from 0 to 0.4 per cent were made up and frozen. Two of the mixes, one containing no egg yolk and one containing 0.4 per cent egg yolk, were frozen fresh; whereas the other 4 mixes were frozen after aging twenty-four hours. The freezing data relative to this group of mixes are expressed in table 2.

It will be noted from the data recorded in table 2 that mixes of comparatively low total solid content do not show the improvement in whipping quality when egg yolk is used that is shown by the heavier, slower freezing mixes previously studied. Aging improved the whipping qualities of these particular mixes to a much greater extent than did the use of egg yolk.

An examination of the ice cream samples obtained from this group of mixes, with a low serum solid content, showed that egg yolk did improve the texture of these samples. The ice cream samples increased in smoothness in direct proportion to the concentration of egg yolk used. All samples in this group were somewhat watery, were lacking in body and did not possess a texture which would compare favorably with the heavier mixes previously studied. The importance of serum solids upon the body and texture of the finished ice cream was brought out very emphatically by an examination of the samples obtained from the freezing of these two groups of mixes reported in tables 1 and 2, respectively.

TABLE 2

Effect of egg yolk of overrun and freezing time of fresh and aged mixes calculated to contain 12 per cent fat, 8 per cent serum solids, 15 per cent sugar, and 0.4 per cent gelatine

Brine temperature + 4°F.

FREEZING TIME	PER CENT OVERRUN					
	Fresh, no egg	Fresh, 0.4 per cent egg	Aged, no egg	Aged, 0.2 per cent egg	Aged, 0.3 per cent egg	Aged, 0.4 per cent egg
minutes						
2	20	19	21	25	21	17
3	25	20	25	28	22	20
4	31	22	32	35		30
5	37	29	43	42	37	43
6	42	39	50	51	47	53
7	47	47	62	57	58	65
8	51	58	69	70	70	76
9	56	66	78	80	82	86
10	65	70	86	90	95	96
11	73	75	89	100		
12	83	81	97			
13	85	84				
14	87	85				
15	88	91				
16	91					
17	92					

The composition of another group of mixes was 12 per cent fat, 13 per cent sugar, 10 per cent serum solids, and 0.4 per cent gelatine and egg yolk varying from 0 to 0.4 per cent. The freezing data relative to this group of mixes are expressed in table 3.

All the mixes in this group were aged twenty-four hours prior to freezing.

The data show that egg yolk had practically no influence on the time or overrun with this group of mixes. These results further substantiate the data recorded in table 2 in showing that the whipping qualities of mixes which naturally freeze and whip readily are improved but little by the addition of egg yolk.

TABLE 3

Effect of egg yolk on overrun and freezing time of mixes calculated to contain 12 per cent fat, 10 per cent serum solids, 13 per cent sugar, and 0.4 per cent gelatine

Brine temperature + 4°F.

FREEZING TIME	PER CENT OVERRUN			
	Aged, no egg	Aged, 0.2 per cent egg	Aged, 0.3 per cent egg	Aged, 0 4 per cent egg
minutes				
2	23	28	24	21
3	31	33	29	27
4	42	40	38	38
5	51	48	49	48
6	56	55	55	55
7	60	59	61	60
8	63	63	64	67
9	71	71	71	74
10	82	78	79	81
11	87	88	88	91
12	92	92	94	101
13	97	101	105	

The body and texture of the ice cream samples obtained from this group of mixes were decidedly improved by egg yolk. The smoothest and most desirable texture was present in the sample of ice cream containing 0.4 per cent egg yolk.

In the opinion of the writers egg yolk should never be used to correct an improperly balanced mix; nor should it be used to correct a mix deficient in solids. A mix of suitable composition must be used if a high quality product is to be produced regardless of whether egg yolk is used or not.

To emphasize the fact that egg yolk is not a substitute for sufficient solids in the mix, freezings were made with a group of mixes having a comparatively low total solid content. The composition of the mix selected was 10 per cent fat, 10 per cent serum solids, 13 per cent sugar, 0.5 per cent gelatine, and egg yolk up to 0.6 per cent.

It was thought unnecessary to include the freezing data for this group of mixes since the data showed only a slight advantage in freezing time in favor of the mixes containing egg yolk; however, some interesting observations were made as regards the body and texture of the finished ice cream.

Fresh mixes from this group were frozen and it was found that egg yolk was extremely beneficial in preventing the watery condition ordinarily encountered with mixes of this composition when frozen fresh. The check mix containing no egg yolk, when drawn from the freezer, melted down rapidly at the sides of the can and brick pans, a condition that would naturally tend to make a coarse, icy product after hardening. Mixes of the same composition, frozen under the same conditions but containing 0.4 per cent egg yolk did not show this watery condition when drawn from the freezer. It should be made clear that a mix of this composition containing as much as 0.6 per cent egg yolk was very inferior from the standpoint of body and texture to the higher solid mixes containing no egg yolk.

The mixes that have been reported thus far, except those given in table 3, have been more or less extreme from the standpoint of composition; hence a group of mixes were frozen that would more nearly represent an average composition. The composition selected was 12 per cent fat, 10 per cent serum solids, 15 per cent sugar, 0.4 per cent gelatine, and 0 to 0.5 per cent egg yolk. A mix of this composition will result in a product of high quality if properly frozen and hardened whether egg yolk is present or not. The freezing data for this group of mixes are expressed in table 4.

It will be noted from table 4 that the greatest saving in freezing time is effected when egg yolk is used with the fresh mixes. There was a saving of 4 minutes resulting from the use of egg yolk with the fresh mix, whereas there was a saving of only 2 minutes after the mixes were aged twenty-four hours. An examination of the ice cream samples from this group of mixes showed that there was very little if any improvement in the body and texture of the samples resulting from the use of egg yolk. It is questionable whether the use of egg yolk can be justified from the improvement that will result in the body and texture of the finished product of aged mixes containing more than 37 per cent total solids.

TABLE 4

Effect of 0.5 per cent egg yolk on the overrun and freezing time of a fresh and aged mix containing 12 per cent fat, 10 per cent serum solids, 15 per cent sugar, and 0.4 per cent gelatine

Brine +5°F. on fresh and +2°F. on aged mix

FREEZING TIME	PER CENT OVERRUN			
	Fresh, no egg	Fresh, 0.5 per cent egg	Aged, no egg	Aged, 0.5 per cent egg
minutes				
2		20	26	24
3	35	23	31	28
4	37	30	38	35
5	41	38	46	42
6	45	46	52	50
7	48	55	58	58
8	52	63	62	65
9	58	67	66	73
10	62	75	73	80
11	66	78	79	84
12	75	80	86	95
13	78	81	89	
14	80	89	96	
15	79			
16	85			
17	87			
18	89			

FRESH EGGS CAN BE SUBSTITUTED FOR DRIED YOLK

Several trials in which fresh whole yolk and fresh whole eggs were substituted for the dried yolk, indicated that just as satisfactory results could be obtained with fresh eggs as with the dried yolk. If fresh eggs of good quality can be obtained it would be desirable in many cases to use the fresh eggs in preference to the dried yolk. The price in most cases is the factor that will limit the use of fresh eggs. If fresh eggs are used allowance must be made for the moisture which they contain in calculating the correct number to use. According to Turnbow and Rafetto (2), about 72 fresh yolks are required to make one pound of dehydrated yolk; this being the case 36 fresh yolks would be required per 100 pounds of ice cream mix to furnish the equivalent of 0.5 pound of dehydrated yolk in this quantity of mix.

SUMMARY

1. The improvement in the whipping qualities of ice cream mixes resulting from the use of egg yolk is dependent upon: (*a*) the composition of the mix; (*b*) the age of the mix, and (*c*) the concentration of egg yolk used.
2. The whipping qualities of relatively heavy, slow freezing mixes are improved to a greater extent by egg yolk than are mixes of comparatively low solid content.
3. Egg yolk facilitates the freezing of fresh mixes irrespective of their composition.
4. The improvement in whipping qualities noted is in most cases in direct proportion to the concentration of egg yolk present.
5. Egg yolk, used in a concentration of 0.5 per cent or less, will exert but little or no influence on the body, texture, appearance, or flavor of ice cream containing sufficient milk solids and sugar.
6. An egg yolk flavor was considered objectionable by many people and it was most apparent in low solid mixes.
7. The results reported in this paper probably apply only when the same type of freezer, same brine temperature, and mixes of the same composition are used that were employed in conducting these experiments.

REFERENCES

(1) Dahle, C. D. 1926 Manual for Ice Cream Makers. Loyless Publishing Co., Atlanta, Georgia.

(2) Turnbow, G. D., and Raffetto, L. A. 1928 Ice Cream. John Wiley & Sons, Inc., New York, N. Y.

(3) Sommer, H. H. 1927 What factors affect whip. Ice Cream Trade Journal, xxiii, no. 8, 64.

(4) Mojonnier, T., and Troy, H. C. 1925 The Technical Control of Dairy Products. Mojonnier Bros. Co., Chicago, Ill.

THE ABILITY OF ESCHERICHIA COLI AND SERRATIA MARCESCENS TO SURVIVE 62.8°C., FOR THIRTY MINUTES IN MILK*

FRED W. TANNER AND MARY F. WINDSOR

Department of Bacteriology, University of Illinois, Urbana

The significance of *Escherichia coli* as an indicator of pollution of water has caused enquiry to be made with regard to the possibility of its use as an indicator of undesirable conditions in other fields. It has been proposed at times that, since it is a non-sporulating species, it might serve as an indicator of efficient pasteurization. Such a use of the organism requires that careful studies be made with regard to the heat resistance of the species in milk before it can be used in such a manner.

HISTORICAL

A survey of the literature of the subject leaves one in doubt with regard to the pertinence of some contributions. In some cases, too little information is given with regard to the organisms used, the technic employed and, if data collected with commercial pasteurizers are reported, the type of machine used. Ayers and Johnson (3) tested the behavior of *Escherichia coli* at pasteurization temperatures. They used 174 strains isolated from human and bovine sources and from dairy products. They found that as a general rule these organisms have a low majority thermal death point, but that resistant strains occurred which survived heating for 30 minutes at 62.8°C. At 60°C. slightly over 50 per cent of the cultures remained viable; at 62.8° only 6.89 per cent survived. The authors, in conclusion, stated that 62.8° was a critical temperature for *Escherichia coli*, but that heating to 65.5°C. should certainly destroy it. In view of these results they advanced the opinion that the presence of *Escherichia coli* in pasteurized milk which has been held for some time is not an indication of inefficient processing but of the multiplication of the few cells which survived. However, large numbers of the

---

* Received for publication November 9, 1928.

organisms present in milk immediately following pasteurization is evidence of inefficient and insufficient heating. Shippen (9) investigated pasteurized and fine quality raw milks in Baltimore. He isolated from pasteurized milks 31 strains of organisms of the *Escherichia coli* type. These were subjected to a temperature of 60°C. for 15 minutes in sealed glass tubes; the survivors, identified by their action when inoculated into sterile litmus milk and then into lactose broth, were heated at 61°C. for 15 and 30 minutes and so on by increasing degrees of heat until they no longer survived. Cultures of the strains killed at the initial temperature were subjected to decreasing degrees of heat until they survived. Of the 31 strains 11 remained viable after 60°C. for 15 minutes; of these, one was killed only at 68°C. in 15 minutes and at 65° for 30 minutes. No culture of *Aerobacter aerogenes* resisted treatment at 60° for 15 minutes. These results confirmed the opinions of workers cited above that there are found resistant strains of *Escherichia coli.* Shippen concluded that the presence of *Escherichia coli* in pasteurized milk is not to be interpreted as an index of improper pasteurization nor of subsequent contamination.

Finkelstein (5) also found 62.8°C. to be a critical temperature for *Escherichia coli* in milk. Since Brown and Gott (4) had stated that Endo's medium could be used to detect *Escherichia coli* in milk and the presence of this organism indicated undesirable conditions, Adams and Ward (1, 2) examined certified and pasteurized milk in Detroit and found that many of the lactose fermenters which appeared on Endo's medium belonged to the coli section of the colon-aerogenes group. Prompted by the work of Twiss, Tanner and Dubois (11) observed the effect of heat on several organisms of the colon-typhoid group among which was a strain of *Escherichia coli.* The strain survived 15 minutes heating under the conditions employed. Seeleman (8), using two strains, found that they were destroyed in less than 30 minutes at from 60 to 63°C. In England, Jenkins (6) stated that an effectively pasteurized milk should not contain *Escherichia coli* and that the organism was a valuable index of pasteurization efficiency.

Swenarton (10) studied the *Escherichia coli* content of milk from 16 pasteurization plants. A satisfactory pasteurization temperature was considered to be 143°F., for 30 minutes with prompt cooling to 40°F. Swenarton suggested the following standard. Five quantities each of 0.1 cc., to be examined and of these not more than 20 per cent to show the presence of members of the *Escherichia coli* group. He further modified the test four times when three or more of the five equal 0.1 cc. portions constituting a single sample showed the presence of these organisms. This was not allowed when it occurred in more than 10 per cent of the standard samples when ten or more were examined, or in one standard sample when less than ten samples were examined.

Leete (7) suggested another organism also as an indicator of efficient processing of milk—*Serratia marcescens* (*Bacillus prodigiosus*). This organism would appear to be very satisfactory, for it is readily cultivated on ordinary media, is easily recognized by its bright red colonies, and is not hard to eradicate from pasteurizing apparatus. In New Jersey an investigation carried out by the State Bureau of Food and Drugs, using the "*Serratia marcescens*" method described by Leete, demonstrated that over 90 per cent of the 49 commercial plants examined held the milk for less than 30 minutes at the required pasteurization temperature (61° to 62.8°C.). The plants tested included 42 systems of the "continuous flow" type, where the milk was heated in one vessel and passed to holding vats the outlets of which were automatically controlled, and 7 vat systems of the compartment type, where the milk was heated and held in the same compartment vat.

EXPERIMENTAL

The experiments reported in this paper were made with a delicately controlled oil bath. The suspensions of cells in milk were placed in sterile Bigelow and Esty tubes so commonly used in thermal death point work. The organisms were grown on agar slants for twenty-four or forty-eight hours at 37°C.; the growth from one slant was washed with about 3 cc. of sterile water or physiological salt solution and thoroughly shaken. One cubic centimeter of this suspension was inoculated into an Erlenmeyer flask containing 75 cc. of sterile milk. After shaking the suspension, 2.5 cc. portions were pipetted into Bigelow and Esty tubes which were then sealed. The tubes were placed in the oil at the same time and one removed at regular intervals.

TABLE 1

Longevity of Escherichia coli (Bacterium coli) in milk heated to 62.8°C.

STRAIN NUMBER	SEALED TUBES		OPEN FLASKS		LITMUS MILK TUBES	
	Initial number per cc.	Survival time	Initial number per cc.	Survival time	Initial number per cc.	Survival time
K1	100,000,000	24	1,000,000	3	2,000,000	24
K2	75,000,000	6	50,000	3	800,000	6
K5	Innumerable	33	Innumerable	30	700,000	21
K10	6,000,000	6	50,000	1	1,500,000	1
K18	50,000,000	6	100,000	9	3,000,000	6
K25	5,000,000	30	5,000,000	9	7,000,000	15
K38	50,000,000	30	1,000,000	30	5,500,000	15
W17	20,000,000	12	500,000	9	6,000,000	18
W27	50,000,000	9	1,000,000	3	Innumerable	9
W28	40,000,000	36	2,000,000	21	Innumerable	15
W35	800,000,000	9	5,000,000	9	4,000.000	3
W40	50,000,000	3	700,000	3	5,000,000	9
W56	1.000,000	3	200,000	1	4,000,000	1
W71	200,000,000	1	30,000	3	13,000,000	1
W98	20,000,000	6	1,000,000	3	1,000,000	1
C3	1,000,000	3	250,000	1	1,500,000	1
C8	10,000,000	6	100,000	1	1,500,000	1
S1	40,000,000	6	80,000	1	4,000,000	1
S2	7,000,000	3	50.000	3	5,000,000	1
S3	20,000,000	3	1,000,000	1	6,000,000	1
S4	50,000,000	6	750,000	3	2,500,000	1
S5	25,000,000	15	1,000,000	6	5,000,000	15
S6	10,000,000	1	100,000	1	1,000,000	1

The strains of *Escherichia coli* designated with the letter K were from the human intestine, those with the letter S from sewage and those with W from water. The cultures of *Serratia marcescens* came from the American Type Culture Collection.

With the technic used, practically all of the twenty-three cultures of *Escherichia coli* (*Bacterium coli*) were killed in 24 minutes or less, in spite of the fact that they were present in numbers much greater than ordinarily occur in milk. The results are given in table 1. Notwithstanding the great variation in concentrations or organisms, no regular relation could be observed between the time of survival and the number of organisms present.

Since *Serratia marcescens* (*Bacillus prodigiosus*) has been proposed by Leete as a useful indicator organism, its behavior in milk at pasteurization temperatures was made a part of this investigation.

TABLE 2

Longevity of Serratia marcescens (Bacillus prodigiosus) when heated in milk at 62.8°C. for thirty minutes

STRAIN NUMBER	SEALED TUBES		OPEN FLASKS	
	Initial number	Survival time	Initial number	Survival time
60	150,000,000	3	380,000	1
174	75,000,000 75,000,000	1 1	200,000,000	1
181	Innumerable 500,000	1 1	4,000,000	6
264	45,000,000 5,000,000	1 1	100,000,000	2
268	100,000,000 100,000,000	1 1	1,000,000	1
274	100,000 500,000	1 1	100,000	2
989	10,000,000 10,000,000	1 1	150,000,000	2
990	4,000,000 4,000,000	1 1	40,000,000	9

The results of the experiments with *Serratia marcescens* are shown in table 2. In the sealed Bigelow and Esty tubes *Serratia marcescens* was killed in a very short time, less than three minutes in every instance except one. The longest survival time in the open flasks was 9 minutes even with a heavy inoculation.

TABLE 3

Longevity of spore forming lactose fermenting bacteria in milk at 62.8°C.

STRAIN NUMBER	INITIAL NUMBER OF CELLS PER CC.	SURVIVAL TIME
		minutes
108	1,000,000	36
	50,000,000	36
141	5,000,000	18
	6,000,000	36
203	Too many to count	3
	10,000,000	3
216	2,000,000	3
	2,000,000	3

TABLE 4

Further tests with strains of Escherichia coli (Bacterium coli) found to resist 62.8°C. for longer than thirty minutes

STRAIN NUMBER	INITIAL NUMBER OF CELLS PER CC.	SURVIVAL TIME
		minutes
K5	Innumerable	33
	24,000,000	12
	100,000,000	24
	7,000,000	18
K25	5,000,000	30
	20,000,000	15
	Innumerable	21
	35,000,000	30
K38	50,000,000	30
	220,000,000	30
	100,000	24
	25,000,000	21
W28	600,000,000	33
	40,000,000	36
	75,000,000	36
	100,000,000	48

Since there are lactose fermenting sporulating bacteria which have been confused with *Escherichia coli*, a few tests were made with them. The results are shown in table 3. Several of these strains were sufficiently resistant to survive 30 minutes heating.

For the open flask experiments according to the method of Twiss whole milk was sterilized and inoculated with about 0.2 cc. of a 24-hour culture. The flask was immersed to the neck in the oil-bath and 1 cc. portions removed at regular intervals. Results are shown in table 1.

Tests were also made following the technic of Ayers. Sterile litmus milk tubes were inoculated and submerged in the oil bath. At regular intervals a tube was removed, chilled in ice water and incubated at 37°C. Results are shown in table 1.

The experiments up to this point showed that among the 23 strains of *Escherichia coli* used there were several which survived 30 minutes exposure to a temperature of 62.8°C. in milk. Since the organism does not form spores, such a resistance to heat is unexpected. The four resistant strains were then examined again in different concentrations of cells. Results are shown in table 4.

CONCLUSIONS

The foregoing experiments on cultures of *Escherichia coli* (*Bacterium coli*) show that most of the strains are destroyed by heating at 62.8°C. for less than 30 minutes. The four cultures found in one or more tests to withstand 30 minutes were isolated either from feces or from water condemned for drinking purposes. All the strains from sewage and from commercially pasteurized milk were found to be killed in 15 minutes or less. Beyond this there seems to be no correlation between source and resistance of the cultures.

The markedly high death rate of *Serratia marcescens* (*Bacillus prodigiosus*) at pasteurization temperatures is regarded by the authors as a serious objection to the use of this organism. It is true that the recovery of the organism from milk which had been passed through a pasteurizer would indicate insufficient heating. On the other hand, failure to find the organism, on the basis of data reported in this paper, might not indicate a heating period much longer than ten minutes, a period too short to yield safe milk. It is probably impossible to secure an organism which will satisfy all of the requirements.

SUMMARY

Of the 23 cultures of *Escherichia coli* (*Bacterium coli*) studied only one was found repeatedly to withstand heating in sealed tubes for 30 minutes at 62.8°C., the temperature of experiment. This one strain was found to survive only 21 and 30 minutes in open containers.

Of the 8 strains of *Serratia marcescens* (*Bacillus prodigiosus*) tested none survived heating for more than nine minutes by any of the methods used.

Of the four sporous, lactose-fermenting forms two were found very resistant both in twenty-four hours and in seven-day old cultures; the other two strains were destroyed in three minutes in both cases. The strain which formed spore-like structures after a few days' incubation was no more resistant in a week-old culture than in a twenty-four hour culture, being killed within three minutes at 62.8°C. in either case.

On the whole, results by the three methods of treatment—in sealed tubes, in open flasks, and in litmus milk tubes—checked fairly closely. The actual time of survival was in most cases shorter in the open flasks than in the sealed tubes. No constant relation could be observed between the concentration of organisms and the time of survival.

Although neither the *Escherichia coli* (*Bacterium coli*) cultures nor the *Serratia marcescens* (*Bacillus prodigiosus*) strains used proved to be very good as indicators of efficiency of pasteurization by the holder process, for several reasons the *Escherichia coli* (*Bacterium coli*) would probably prove to be more accurate as such an indicator. It seems fair to assume, in view of the results obtained, that *Escherichia coli*, in concentrations in which it occurs in milk, would be destroyed by exposure to 62.8°C. for 30 minutes, yet there is always the possibility of encountering resistant strains or cultures containing some resistant cells.

REFERENCES

(1) Adams, F. O., and Ward, A. R. 1925 Does Endo's medium locate milk contamination? Amer. Food Jour., xx, 147–148.

(2) Adams, F. O., and Ward, A. R. 1924 Tests of certified and pasteurized milk for *B. coli*-like colonies on Endo's medium. Report International Association Dairy and Milk Inspectors, 249–251.

(3) Ayers, S. H., and Johnson, W. T. 1915 Ability of colon bacilli to survive pasteurization. Jour. Agr. Res., iii, 401–410.

(4) Brown, L. A., and Gott, E. J. 1923 The use of Endo's medium in locating milk contamination. Amer. Food Jour., xviii, 295–296.

(5) Finkelstein, R. 1919 Occurrence of colon-aerogenes group in raw and pasteurized milk and its significance. Jour. Dairy Sci., ii, 460–481.

(6) Jenkins, Hermina. 1926 Experiments on the pasteurization of milk, with reference to the efficiency of commercial pasteurization. Jour. Hyg., xxv, 273–284.

(7) Leete, C. S. 1923 Continuous flow holders used in pasteurization, especially in regard to the time factor, from a bacteriologist viewpoint. Proc. World's Dairy Cong., ii, 1212–1216.

(8) Seelemann, M. 1926 Uber die Widerstandkraft der Bakterien der Typhus-Paratyphus Gruppe in dauererhitzen Milch. Zeitschr. f. Fleisch- und Milchhygiene, xxxvi, 250–256.

(9) Shippen, L. P. 1915 Significance of Bacillus coli in pasteurized milk. Jour. Amer. Med. Assoc., lxiv, 1289–1291.

(10) Swenarton, J. C. 1927 Can *B. coli* be used as an index of the proper pasteurization of milk? Jour. Bact., xiii, 419.

(11) Tanner, F. W., and Dubois, G. C. 1925 Some notes on the effect of heat on members of the colon-typhoid group in milk. Jour. Dairy Sci., viii, 47–53.

SOME FACTORS AFFECTING THE FAT CLUMPING PRODUCED IN MILK AND CREAM MIXTURES WHEN HOMOGENIZED*

F. J. DOAN†

Department of Dairy Husbandry, Pennsylvania Experiment Station, State College, Pennsylvania

That homogenization of cream and ice cream mix leads in most cases to a clumping of the small fat globules produced by the process, is quite well established. Mortensen (1), Martin and Dahle (2), Sherwood and Smallfield (3), and Reid and Moseley (4) have published accounts of experimental work wherein this fat-clumping phenomenon has been definitely noted. These investigators also interpret differences in viscosity between similarly processed products as being due to differences in the degree of such clumping or to variations in the size of the clumps formed. The writer (5) found an explanation for the enhanced creaming ability of the so-called "viscolized milk" in the fact that such milk contains clumps of small fat globules produced by the viscolizer or homogenizer. Reid (6) in a later report further states that the clumping effect is not influenced by changes of the pressure of homogenization. Workers at the Iowa Station (7) report a substantiation of this finding and in addition assert that clumping is not affected in any definite manner by the fat content of the cream and ice cream mixes processed. Very recently Hening (8) has found that the presence of sugar in ice cream mixes at the time of homogenization increases the extent of fat-clumping compared with that exhibited by mixes processed prior to the addition of sugar. He also notes the fact that the fat in milk mixtures containing low percentages of fat, tends to clump less than in mixtures of high fat content. Webb and Holm (9) also recognize the factor of fat-clumping and in their study of cream feathering suggest that the phenomenon may be due to changes of potential at the fat globule-plasma interface.

---

* Received for publication November 19, 1928. Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper No. 461.

† Assistance in preparing and processing many of the mixtures used was rendered by H. V. Green.

The study reported here was undertaken in an effort to learn more about the factors playing a part in the fat-clumping phenomenon produced by the homogenizer. Several contradictory findings are reported in the literature and the actual mechanism of the clumping is almost a complete mystery which can only be understood when all the possible factors playing a part have been thoroughly investigated. The subject is a very complex one. The findings here set forth are offered as a further contribution to existing knowledge and in no sense as an exhaustive investigation of the subject.

EXPERIMENTAL

General methods

In all cases, except as specifically noted, only fresh, unpasteurized cream, milk, and skimmilk were used in this study. The mixtures were prepared from cream standardized with milk or skimmilk to the desired composition as near as could conveniently and quickly be done after which in most cases the exact analysis for percentage of fat and plasma solids was obtained by standard Mojonnier methods, although in a few cases where fat only was determined, the Babcock method was employed. The study was made only on mixtures ranging in fat content from about 3 to 16 per cent. A Union Steam Pump Company Viscolizer of 250 gallons per hour capacity was used in all cases, special precautions being taken to assure accurate pressures and accurate samples of the processed mixtures. In all cases, unless otherwise stated, the mixtures were processed immediately at 38°C. (100°F.) to which point they were quickly warmed after standardizing and were subjected to a pressure of 3500 pounds.

Microscopic methods

It was realized at once that some uniform method of examining samples for the comparative degree of clumping of the fat was necessary. Considerable time was devoted to a study of this phase of the investigation using microscopic methods entirely. The effects of various dilutants and methods of diluting, the relative value of hanging drops and capillary spaces, as well as a comparison of methods of counting and measuring clumps, were considered carefully.

It was found that dilution with water did not change appreciably the comparative condition of the various samples and since in undiluted samples differences of great magnitude only can be detected, the dilution method of examination was used. The use of the hanging drop or capillary space for microscopical observation were found equally satisfactory but since in the former case the samples are more readily prepared, this method was adopted.

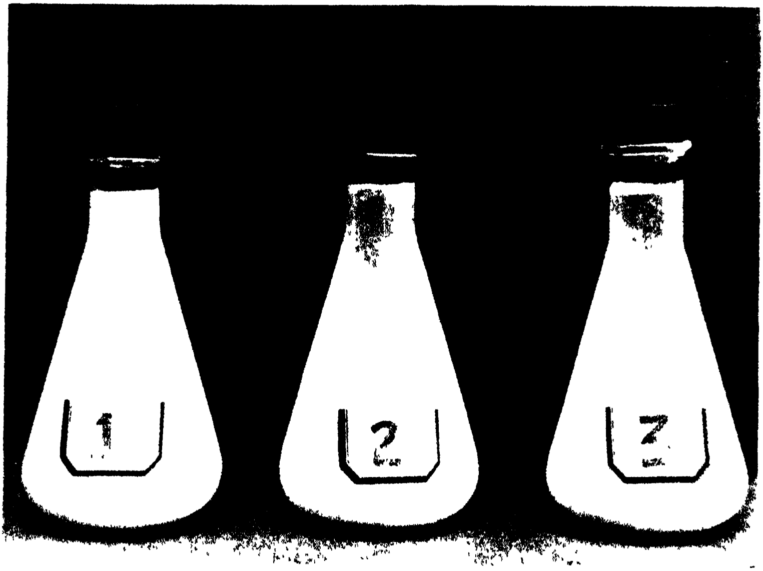


FIG. 1. THE APPEARANCE OF SAMPLES IN ERLENMEYERS

Sample 1. Fat pronouncedly clumped giving a coagulated appearance on the side of container.

Sample 2. Fat-clumping at "threshhold point," side of container has a faint feathered appearance.

Sample 3 Fat not clumped, no unnatural effect on side of container.

These three samples are numbers 1, 2 and 3 respectively in Table 8, series B.

Considerable difficulty was encountered in obtaining a satisfactory method for rating the relative degree of fat-clumping in the various samples. Counts of the number of clumps per field is difficult due to the fact that the field is not a plane surface of one layer and some clumps are partly in and partly out of focus. Furthermore, due to gravity, the clumps tend to rise and fields change continuously, the upper layers becoming progressively more dense with a passage of time.

Measurement of the clumps is also difficult. The total length and greatest width are not entirely satisfactory when volume is what is desired. Furthermore, since large clumps rise more rapidly than small ones, inaccuracies occur depending on the stratum of the drop selected for observation and the time elapsing between preparation and observation.

The method finally used in the experiments is one which establishes what is hereafter designated as the "threshhold point of clumping." This may be defined as the point at which the clumping of the fat is sufficiently in evidence under the microscope to be definitely determined by observation with the low power objective (16 mm. objective and 10× eye piece draw tube down). This point was found to coincide quite closely with the point at which increases in viscosity become easily noted with a pipette and stop-watch. It also lies very near the point at which a feathering of the processed sample becomes visible on the inside glass of the containing vessel (fig. 1).

The microscopic appearance of a sample at the threshhold of clumping is illustrated by figures 2a and 2b. Samples exhibiting a degree of clumping greater than at the threshhold (figs. 3a and

Figs. 2*a* and 2*b*. Microscopic Views of a Sample (No. 2, Table 8, Series B) at the Threshhold of Clumping

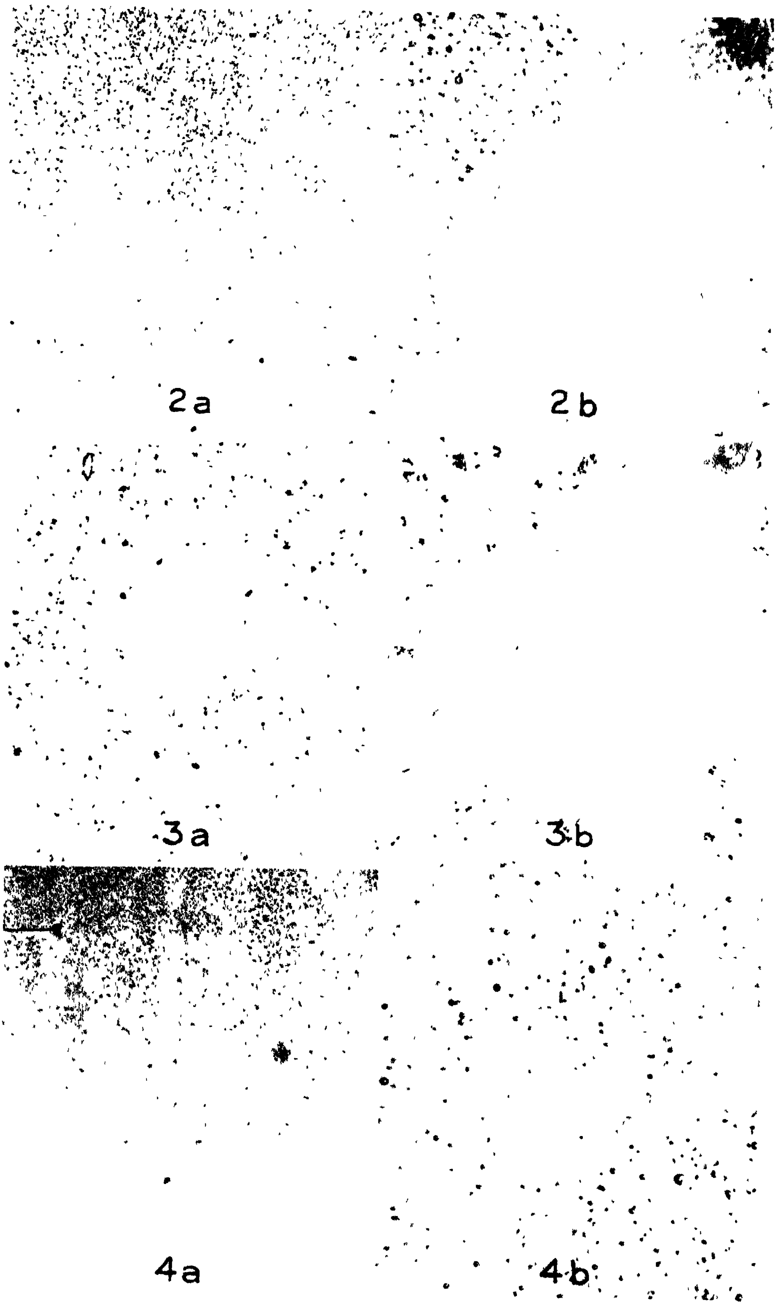
2a. Under low power magnification (16 mm. objective). 2b. Under high power (4 mm objective).

Figs. 3*a* and 3*b*. Microscopic Views of a Sample (No. 3, Table 8, Series B) Exhibiting Fat-clumping to a Greater Degree than at the Threshhold Point

3a. Under low power magnification (16 mm. objective). 3b. Under high power (4 mm. objective).

Figs. 4*a* and 4*b*. Microscopic Views of a Sample (No. 1, Table 8, Series B) Exhibiting Fat-clumping to a Lesser Extent than at the Threshhold Point

4a. Under low power magnification (16 mm. objective). 4b. Under high power (4 mm.). In this case no clumping at all is evident.



FIGS 2 to 4

3b) and lesser than at the threshhold (figs. 4a and 4b) are described in the tables in a comparative manner.

The average size of the clumps at the threshhold point as above defined was found to be 2.96μ by 4.84μ, these values being the average for three different samples which were judged to be at this threshhold. These values however are only approximate due to the difficulties encountered in measuring noted previously.

While this method does not give an extremely sharp separation of the clumped and not clumped samples yet it was nevertheless judged to be best suited for the purpose, inasmuch as only samples in the same series are definitely compared in this study. In one case however the composition of samples at or near the threshhold in several series is compared. The threshhold is a rather definite point in all series.

The dilution of samples in all cases was made at the rate of 1 cc. of sample to 100 cc. of distilled water for a sample containing 8 per cent of fat. For samples of other fat content, the amount of water was varied to give a corresponding amount of fat in the dilution. The dilution was made in a tube and the tube inverted twice after which the hanging drop slide was at once prepared and observed.

Emulsion, stability, and clumping

It has long been recognized that milk or cream subjected to homogenization or viscolization will not separate a cream layer on standing. This fact is found to be true regardless of whether or not the fat is in a clumped or non-clumped condition. If, however, such samples be diluted with skimmilk or normal whole milk, creaming takes place very readily, the amount of cream forming being greater as the degree of fat-clumping is more pronounced, although as would be expected, samples containing unclumped fat diluted with skimmilk form no cream layer.

Table 1 gives data showing these effects as obtained in one characteristic series of several that were studied. In this series six samples of various fat content, ranging from 4.2 to 16.1 per cent were homogenized. They were examined microscopically and portions were allowed to stand in cylinders for 48 hours at 10°C. (50°F.) for observations as to creaming ability.

It will be noted that while variations in the degree of fat-clumping ranged from none at all to pronounced clumping, none of the samples gave evidence of any creaming tendency whatever nor did the upper layers in the cylinders analyze appreciably higher in fat content. When, however, the samples were diluted with raw skimmilk to a uniform fat content of 3 per cent, pronounced creaming occurred where fat clumps were present, the depth of the layer increasing with the degree of clumping. It will also be noted that the previously defined threshhold point of clumping lies about at the point where the creaming tendency on dilution becomes evident.

TABLE 1

Clumping and stability of homogenized mixtures. Homogenization pressure 3500 pounds, temperature 38°C. (100°F.)

Undiluted

SAMPLE	FAT	MICROSCOPIC APPEARANCE OF FAT	CREAM LAYER —48 HOURS AT 10°C.	FAT IN UPPER LAYER OF CYLINDER
	per cent		*per cent*	*per cent*
1	4.2	Well dispersed—no clumps	None	4.3
2*	7.2	Clumping tendency	None	7.15
3*	9.5	Evident clumping	None	9.7
4	11.0	Very evident clumping	None	11.0
5	13.5	Prominent clumping	None	13.6
6	16.1	Pronounced clumping	None	16.3

Diluted

SAMPLE	CREAM LAYER—48 HOURS AT 10°C.
	per cent
1, plus skimmilk to 3 per cent fat	0.0
2, plus skimmilk to 3 per cent fat	0.0
3, plus skimmilk to 3 per cent fat	16 0
4, plus skimmilk to 3 per cent fat	20.5
5, plus skimmilk to 3 per cent fat	39.0
6, plus skimmilk to 3 per cent fat	68.0†

* Indicates position of threshold point of clumping in the series—as defined previously.

† Line indistinct.

Effect of fat concentration on clumping

The data in table 1 indicates that when other things are constant, the degree of fat-clumping increases with the concentration of fat in the mixtures. In further support of this fact, data is given in table 2 showing 4 series of processed samples, in each one of which the samples were identical in all respects except fat

TABLE 2

The increase in clumping with fat content. Homogenization pressure 3500 pounds, temperature 38°C. (100°F.)

FAT IN MIXTURE	MICROSCOPIC APPEARANCE
Series 1	
per cent	
8.0	Well dispersed—no clumps
9.5	Evident clumping
10.5*	Very evident clumping
14.0	Pronounced clumping
Series 2	
9.4	Clumping not evident
10.6*	Evident clumping
11.4	Very evident clumping
12.6	Prominent clumping
Series 3	
11.8	Very evident clumping
12.8	Very prominent clumping
13.9	Pronounced clumping
Series 4	

Fat	Plasma solids	Plasma solids/fat ratio	MICROSCOPIC APPEARANCE
per cent	*per cent*		
12.91	8.40	0.650	Very evident clumping
13.80	8.35	0.605	Prominent clumping
14.18	8.11	0.572	Pronounced clumping
15.02			Very pronounced clumping

* Indicates position of the threshold point of clumping in the series.

content and were homogenized and treated in identical fashion. The fat content unquestionably is an important factor in the clumping of the small globules produced during homogenization, especially when the mixture is not far removed from the threshhold point. The fact that other investigators (7) have not found fat content to affect the degree of clumping, must be due to the fact that their investigations were concerned with samples showing very pronounced clumping tendencies wherein only differences of great magnitude could readily be discerned. The results given here are in agreement with the findings of Hening (8) who states that mixtures containing between 5 and 10 per cent of fat clump little but who found that mixtures containing higher percentages of fat clumped very markedly when homogenized.

Effect of homogenizing pressure

It is logical to suppose that the pressure used in the homogenizing process would materially affect the tendency of the fat globules to clump, at least pressures up to such a point that increases would not further subdivide the globules. Very low pressures do not give efficient homogenization and therefore clumping would be expected to be diminished compared with what would obtain for higher pressures. This was found to be the case in a number of trials only two of which are here reported in table 3. It is not felt that these results need comment even though two pieces of research previously referred to (6) (7) are not in agreement in the matter. Here again the lack of substantiation must be due to the fact that in the previous work the mixtures used were too far removed from the threshhold of clumping or the methods of comparing the degree of clumping unsuited to the condition of the samples. It might be mentioned that the results shown in table 3 illustrate the effect of fat concentration in a somewhat different manner. Sample 2 containing 14.09 per cent of fat arrived at the threshhold point at 2000 pounds homogenization pressure whereas sample 1 with only 12.51 per cent of fat required 3000 pounds to produce the same result.

TABLE 3

The effect of homogenizing pressure on clumping. Temperature 38°C. (100°F.)

PRESSURE USED (HOMOGENIZATION)	MICROSCOPIC APPEARANCE
Sample 1. 12.51 per cent fat; 8.04 per cent plasma solids; plasma solids/fat = 0.643	
pounds	
1000	Homogenization poor—no clumps
2000	Homogenization fair—no evident clumps
3000*	Homogenization fair—evident clumps
4000	Homogenization good—very prominent clumps
Sample 2. 14.09 per cent fat; 7.86 per cent plasma solids; plasma solids/fat = 0.558	
1000	Homogenization poor—no clumps
2000*	Homogenization fair—evident clumps
3000	Homogenization good—very prominent clumps
4000	Homogenization good—very pronounced clumps

* Indicates position of the threshold point of clumping in the series.

TABLE 4

The effect of heated plasma on clumping. Homogenization pressure 3500 pounds, temperature 38°C. (100°F.)

Analysis of samples

SAMPLE	FAT	PLASMA SOLIDS	PLASMA SOLIDS/FAT RATIO	COMPOSED OF
	per cent	*per cent*		
1	11.80	7.72	0.654	Raw cream + raw skim
2	12.13	7.83	0.646	Raw cream + heated skim
3	13.04	7.69	0.590	Raw cream + raw skim
4	13.05	7.83	0.600	Raw cream + heated skim

Condition of fat after homogenizing

SAMPLE	MICROSCOPIC APPEARANCE
1	Homogenization good—pronounced clumping
2	Homogenization good—evident clumping
3	Homogenization good—very pronounced clumping
4	Homogenization good—prominent clumping

Effect of heating the plasma

It is a well recognized fact that heating milk destroys the creaming ability. Palmer and Anderson (10) have shown that this effect is even more pronounced when the plasma alone is heated and since later work by Troy and Sharp (11) and Orla Jensen (12) have quite definitely indicated that creaming ability is dependent on the ability of the fat globules to clump and have further shown that heating of milk retards or even destroys clumping, it is a natural conclusion to believe that the heating of the plasma prevents the clumping.

TABLE 5

The effect of developed acidity on clumping. Homogenization pressure 3500 pounds, temperature 38°C. (100°F.)

SAMPLE		ACIDITY	MICROSCOPIC OBSERVATION (AFTER HOMOGENIZATION)
		per cent	
1	Raw	0 09	Prominent clumping
	Fermented	0.28*	Very prominent clumping
2	Raw	0.14	Clumping barely evident
	Fermented	0.25	Clumping barely evident
3	Raw	0.12	Evident clumping
	Fermented	0 26	Barely evident clumping
4	Raw	0.11	Pronounced clumping
	Fermented	0.25	Very prominent clumping

* Slight precipitation of casein in this sample after homogenizing.

Experiments were made to ascertain if this same effect could be noted in the clumps produced by the homogenizer. A portion of fresh raw skimmilk was divided into two parts, one of which was heated to 82.2°C. (180°F.) for 10 minutes and cooled. Fresh raw cream containing 53.4 per cent fat was then standardized to approximately 12 per cent fat using the raw skimmilk and again using the heated skimmilk. Two other mixtures of approximately 13 per cent fat were compounded in the same way. These four mixtures were then warmed and homogenized as usual. Under the microscope reduction in the degree of clumping was plainly evident in the two samples containing the heated plasma. Data obtained with these samples is given in table 4.

This experiment was repeated twice with substantially the same results. It might be mentioned that Hening (13) in a very recent report shows that high temperature pasteurization of ice cream mixes (180°F. for 10 minutes) considerably diminishes the size and number of fat clumps produced by the homogenizer.

TABLE 6

The effect of the plasma solids/fat ratio on clumping. Homogenization pressure, 3500 pounds, temperature 38°C. (100°F.)

Four per cent fat mixtures

SAMPLE	FAT	PLASMA SOLIDS	PLASMA SOLIDS/FAT	MICROSCOPIC OBSERVATION
				Series A
	per cent	*per cent*		
1	3.22	0.66	0.170	Homogenization good—evident clumps
2*	3.74	1.54	0.406	Homogenization good—evident clumps
3	3.94	2.31	0.586	Homogenization good—clumps not evident
4	3.94	3.23	0.820	Homogenization good—no clumps
5	4.01	3.98	0.993	Homogenization good—no clumps
				Series B
1	4.00	0.88	0.220	Homogenization good—evident clumps
2*	4.08	1.67	0.409	Homogenization good—evident clumps
3*	3.93	2.37	0.603	Homogenization good—clumps not evident

* Indicates threshold point of clumping in the series.

Effect of acidity

If electrical effects at the fat globule-plasma interface are to any great extent responsible for the fat-clumping phenomenon as has been suggested by Webb and Holm (9), hydrogen ion concentration or acidity variations should cause differences in the degree of clumping. To note the influence of changes in acidity, a portion of four mixtures, exhibiting, when fresh, considerable difference in the degree of clumping produced by the homogenizer, were allowed to ferment slightly and were homogenized in identical fashion to the unfermented portions. The two portions of the same samples were then compared. The results constitute table 5 and are difficult to interpret in a positive way. Apparently acidity has little effect—too little to be shown by the method employed. This was somewhat of a surprise as it was confidently expected that variations in the acidity (pH) would very noticeably affect the ability of a given sample to form clumps during homogenization. The results obtained in these trials suggest the probability that the electrical charge on the fat globule plays little part in the clumping phenomenon since the electrical charge of colloids and emulsions is materially affected by changes in the hydrogen ion concentration of the dispersed phase. The results obtained with the four samples are shown in table 5. It will be noted that sample 1 was the only sample that gave evidence of any increased tendency to clump with increased acidity and in this case there was a slight but evident coagulation of the casein.

TABLE 7

The effect of the plasma solids/fat ratio on clumping. Homogenization pressure 3500 pounds, temperature 38°C. (100°F.)

Eight per cent fat mixtures

SAMPLE	FAT	PLASMA SOLIDS	PLASMA SOLIDS/ FAT	MICROSCOPIC OBSERVATION
				Series A
	per cent	*per cent*		
1	8.31	3.18	0.382	Homogenization good—prominent clumps
2	8.25	4.91	0.595	Homogenization good—very evident clumps
3*	8.28	5.72	0.691	Homogenization good—evident clumps
4			0.800†	Homogenization good—no clumps
5	7.78	8.29	1.065	Homogenization good—no clumps
				Series B
1	7.94	3.44	0.433	Homogenization good—prominent clumps
2*	7.85	5.91	0.752	Homogenization good—evident clumps
3*	7.81	7.93	1.014	Homogenization good—no clumps

* Indicates threshold point of clumping in the series.

† Ratio desired.

TABLE 8

The effect of plasma solids/fat ratio on clumping. Homogenization pressure, 3500 pounds, temperature 38°C. (100°F.)

Twelve per cent mixtures

SAMPLE	FAT	PLASMA SOLIDS	PLASMA SOLIDS/ FAT	MICROSCOPIC OBSERVATION
				Series A
	per cent	*per cent*		
1	11.03	5.96	0.540	Homogenization good—very prominent clumps
2	11.37	7.82	0.688	Homogenization fair—evident clumps
3*	11.82	10.07	0.852	Homogenization good—evident clumps
4	11.78	12.29	1.043	Homogenization good—no clumps
				Series B
1	11.90	5.73	0 482	Homogenization good—pronounced clumps
2*	12.04	8.87	0.737	Homogenization good—evident clumps
3	12.30	12.47	1 014	Homogenization fair—no clumps
4	12.88	17.07	1 325	Homogenization fair—no clumps

* Indicates threshold point of clumping in the series.

TABLE 9

The effect of the plasma solids/fat ratio on clumping. Homogenization pressure, 3500 pounds, temperature 38°C. (100°F.)

Sixteen per cent mixtures

SAMPLE	FAT	PLASMA SOLIDS	PLASMA SOLIDS/ FAT	MICROSCOPIC OBSERVATION
				Series A
	per cent	*per cent*		
1	15.60	6.78	0.434	Homogenization good—pronounced clumps
2	15.50	9.95	0.643	Homogenization good—prominent clumps
3*	15.93	12.72	0.798	Homogenization good—evident clumps
4	16.13	16 12	0.999	Homogenization good—no clumps
				Series B
1	15.26	8.10	0.531	Homogenization fair—pronounced clumps
2*	15.47	11.56	0.747	Homogenization good—evident clumps
3*	15.55	15.37	0.988	Homogenization good—no clumps
4	15.43	18.86	1.222	Homogenization good—no clumps

* Indicates threshold point of clumping in the series.

Effect of amount of plasma solids

As has already been shown, high fat mixtures give evidence of clumping under the conditions used; whereas low fat mixtures do not. As a result of studies not given here, it was found that the threshhold of clumping usually appears in a series of normal mixtures (cream and skimmilk or cream and whole milk) under the conditions used, when the fat content reaches about 11 per cent. This suggested the possibility that the amount of plasma

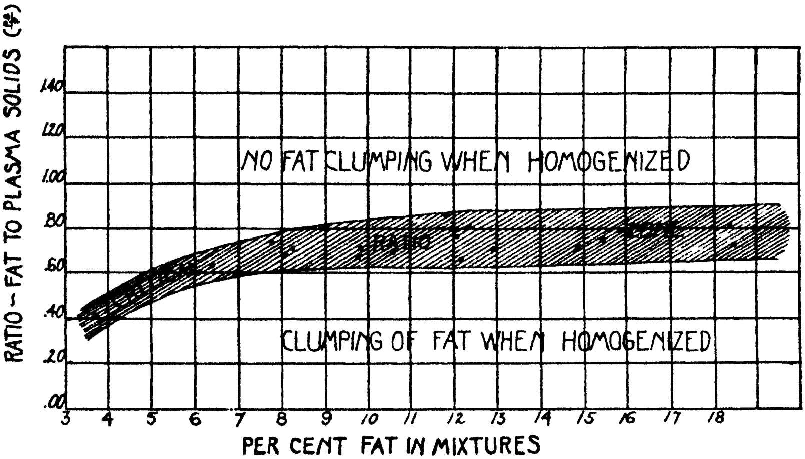


FIG. 5. PER CENT OF FAT IN HOMOGENIZED MIXTURES PLOTTED AGAINST THE PLASMA SOLIDS/FAT RATIO

Dots represent samples judged to be at the threshhold of clumping. The figure shows a zone above which no clumping of fat occurs, but below which the fat globules clump, when mixtures such as were used are homogenized at 3500 pounds pressure and at 38°C. (100°F.).

solids compared with the amount of fat might be an important limiting factor in the fat-clumping phenomenon.

In order to study this phase of the subject, many series of samples were prepared at different fat levels, each sample in the series containing as near as possible the same amount of fat but with varying amounts of plasma solids. The materials used in making the mixtures were raw cream, raw skimmilk, plain condensed skimmilk (manufactured using as low heats as possible) and water where necessary. The mixtures were processed as stated under "methods" in all cases and the samples in each series made as nearly as possible from the same products.

The results of processing two series of samples of approximately 4 per cent fat are shown in table 6, together with the exact analysis of these samples and the plasma solids/fat ratio.

It will be noted that even low fat mixtures can be made to clump if the plasma solids are sufficiently reduced. The critical ratio for clumping in these series appears to be about 0.400 although in other trials the value varied from about 0.400 to about 0.510 due undoubtedly to factors incapable of being accurately controlled, or to physical differences in the products composing the samples in the different series.

Tables 7, 8 and 9 show characteristic data of similar nature obtained from trials at fat levels of approximately eight, twelve and sixteen per cent, respectively. Other series have been omitted for the sake of brevity.

In all some fifty series of samples were prepared and observed from which it was possible to select 19 samples which were deemed to represent the threshhold point of clumping. The fat content of these samples is plotted against the ratio of fat to plasma solids in figure 5 and a zone indicated which represents the critical ratio above which the fat does not clump in homogenized mixtures processed under the conditions used in this study but below which clumping occurs to a very evident extent.

The factor of plasma solids/fat ratio undoubtedly explains why clumping takes place in some ice cream mixes and not in others when the processing is carried out in the same fashion and throws some light on the contrary results obtained by different investigators studying the behavior of ice cream mixes although of course other factors must be considered as well.

DISCUSSION

The clumping tendency exhibited by fat globules is evidence that there is some attraction between them. What this may be is rather problematical but from a review of the existing knowledge, it can probably best be argued that it is an interfacial tension effect.

Fat and water interfaces give rise to relatively high interfacial tensions and since the magnitude of this free energy can be diminished by a decrease in surface area, and a decrease in surface area can be brought about by coalescence of the fat globules into larger units, the globules must exhibit some tendency to thus come together and unite. Indeed in pure water the globules would undoubtedly follow this procedure until ultimately a layer of fat and a layer of water would be formed presenting the smallest interface possible. In milk however there are opposing forces which act to neutralize the inter-globule attraction.

The free energy represented by the fat-globule/water interface may be reduced in another way from that represented by reduction of surface area, namely by the adsorption of surface tension active substances present in the water or in the case of milk, in the plasma. Colloids as a class are capable of lowering energy at surfaces and in milk enough evidence is at hand to practically prove that adsorption of the colloids takes place to a considerable extent. By mathematical means Troy and Sharp (11) have arrived at a thickness of 19mμ for the adsorbed layer although they consider this figure somewhat high. In any case adsorption occurs and the free energy is decreased, although evidently not entirely done away with since the fat globules in normal milk tend to aggregate into clumps due to a slight attraction remaining. However no coalescence takes place, either because the tendency has been so greatly overcome that it is insufficient to do more than attract the globules together or because the adsorbed layer prevents an intimate contact of the globules. The later idea is the more logical.

When milk or cream is homogenized, the normal fat globules are divided into from 10 to 100 individuals. This action creates an enormous new surface area which, of course, represents a considerable increase of interfacial tension and which in turn creates an increased attraction between globules and a great activity in the adsorption of the surface active plasma colloids. If the fat content of the product homogenized is low, the divided globules will be relatively far apart and before any noticeable amount of clumping can occur, the interfacial tension of the globules has been practically neutralized by diffusion of the plasma colloids to the surface and adsorption. If however the fat content of the product is high, the globules are relatively close together and clumping occurs before the adsorption has progressed far enough to greatly reduce the inter-globule attraction. In this case further diffusion of the colloids to the interface and adsorption will be much greater on the outer surface of the clump than on the inner surfaces. This will tend to leave more or less undisturbed the attraction between the globules forming the cluster or clump and may explain the comparative stability of the clumps. It must be assumed that even in the richest creams enough adsorption takes place instantly, from the immediately surrounding plasma to prevent a coalescence of the globules.

It might be inferred that in the case of homogenized milk (low fat content), where no clumpimg is produced, the individual small globules present would clump on aging just as the individual large globules do in normal milk. This is not the case however, and the only apparent explanation seems to be the much greater Brownian movement in the case of the smaller globules which more than overcomes the inter-globule attraction.

The facts brought out regarding the effect of the plasma solids/fat ratio and its affect on the clumping phenomenon can be fitted into the hypothesis set forth above as is more or less evident. Increases in the amount of plasma colloids would mean more available surface active material in the immediate vicinity of the newly formed surfaces after homogenization which would, of course, tend to more quickly overcome the free surface energy created and prevent the attraction between globules from being so great. In such cases clumping would be greatly reduced in amount, if not entirely prevented, depending on the colloid concentration.

The increase of homogenizer pressure was found to favor clumping and since such increases in pressure create smaller globules, more fat surface and a greater total increase in the amount of interfacial tension, the immediately available plasma colloids would be more quickly exhausted without reducing the inter-globule attraction to so low a degree as would occur with lesser pressures.

The writer realizes the futility of attempting to explain such an undoubtedly complex phenomenon as fat-clumping from the standpoint of one effect, and does not intend for this discussion to do so. It is only offered as an interesting probability which seems to fit fairly well the facts brought out in the study of homogenization and if true or partly true other effects must of course not be overlooked.

SUMMARY AND CONCLUSIONS

The following statements seem warranted as a result of facts brought out in this study of the fat-clumping produced in milk and cream mixtures when subjected to homogenization.

1. Homogenized mixtures, regardless of fat-clumping or the absence thereof are stable emulsions, but when diluted with fluid skimmilk or whole milk, cream pronouncedly if fat-clumps are present.
2. The clumping of the small fat globules in homogenized mixtures of normal milk and cream is greatly stimulated by increases in the fat content and also by increases in the pressure used in homogenization.
3. Changes in the acidity of the plasma have little influence on the fat-clumping tendencies exhibited by such mixtures when homogenized.
4. Heated plasma has an inhibiting effect on the production of fat-clumps by the homogenizer.
5. The ratio of the amount of plasma solids to the amount of fat in the mixtures processed is a limiting factor in the fat-clumping phenomenon. There is a critical ratio above which no clumping is obtained but below which clumping is evident or even pronounced.
6. Due to many factors which are difficult or impossible to control, it is not possible to establish a definite value for the critical ratio, since mechanical efficiency of the homogenizer, individuality in the physical properties of different samples of milk, the fat concentration of the mixture and other undefined factors influence the value.
7. The critical ratio appears to be between 0.60 and 0.85 for

mixtures compounded from raw products (with the exception of plain condensed skimmilk), warmed to 38°C. (100°F.), homogenized with a Union Steam Pump Company Viscolizer at 3500 pounds pressure, and of fat concentrations from 8 to 18 per cent.

8. Similarly treated mixtures containing lower concentrations of fat appear to have somewhat lower critical ratios, the values for 4 per cent fat mixtures lying between 0.40 and 0.50.

9. Fat clumping seems to be best explained on the basis of an interfacial tension effect although it is realized that the complete explanation is probably based on more than one effect.

REFERENCES

(1) MORTENSEN, M. 1918 Factors which influence the yield and consistency of ice cream. Iowa Agr. Exp. Sta. Bul. 180.

(2) MARTIN, W. H., AND DAHLE, C. D. 1925 The use of the two stage valve. 38th Ann. Pennsylvania Agr. Exp. Sta. Rpt., pp. 23.

(3) SHERWOOD, F. F., AND SMALLFIELD, H. D. 1926 Factors influencing the viscosity of cream and ice cream. Jour. Dairy Sci., ix, no. 1, 68.

(4) REID, W. H. E., AND MOSELEY, W. K. 1926 The effect of processing on the dispersion of fat in an ice cream mixture. Missouri Agr. Exp. Sta. Res. Bul. 91.

(5) DOAN, F. J. 1927 Viscolized milk and its detection. Jour. Dairy Sci., x, no. 6, 501.

(6) REID, W. H. E. 1927 Homogenization: its effect on physical properties of ice cream. Proceedings, 27th Convention, International Assn. Ice Cream Mfgrs., pp. 103.

(7) MORTENSEN, M., ET AL. 1927 Experiments with dairy products. Iowa Agr. Exp. Sta.Rpt., pp. 31.

(8) HENING, J. C. 1928 Effect of homogenizing ice cream mixes before and after the addition of gelatine or sugar and before and after condensation. Jour. Dairy Sci., xi, no. 4, 299.

(9) WEBB, B. H., AND HOLM, G. E. 1928 The heat stability and feathering of sweet cream as affected by different homogenization pressures and different temperatures of forewarming. Jour. Dairy Sci., xi, no. 3, 243.

(10) PALMER, L. S., AND ANDERSON, E. O. 1926 Physico-chemical factors influencing cream rising. Jour. Dairy Sci., ix, no. 1, 1.

(11) TROY, H. C., AND SHARP, P. F. 1928 Physical factors influencing the formation and fat content of gravity cream. Jour. Dairy Sci., xi, no. 3, 189.

(12) JENSEN, ORLA 1928 A new investigation concerning the low temperature pasteurization of milk and a reaction for controlling pasteurizing temperature. Proceedings Worlds Dairy Congress, Section II, London, England.

(13) HENING, J. C. 1928 Temperature of pasteurization—how it affects the mix properties. Ice Cream Trade Jour., xxiv, no. 10, 53.

THE ANTIRACHITIC VALUE OF IRRADIATED ICE CREAM

WALTER C. RUSSELL, FORREST C. BUTTON, AND
ORME J. KAHLENBERG*

Departments of Agricultural Biochemistry and Dairy Husbandry, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey

The pioneer investigations of Hess (1) and Steenbock (2) and their collaborators have led to numerous studies of the effect of ultraviolet rays on various foods, including milk and milk products, but a search of the literature failed to reveal the results of any special attention to the development of the antirachitic property by the irradiation of ice cream. A study of the vitamins in ice cream was begun in 1922 by Smith (3), who found that vitamin A was present in samples of commercial ice cream, in such concentrations that one could conclude that "no noteworthy alteration in its potency was caused by pasteurizing and freezing." Normal growth in rats was induced by one gram of 10 per cent fat ice cream and ophthalmia cured by 0.25 gram containing 25 mgm. of butterfat. Freezing had no effect on vitamin B. Ice cream made from pasteurized products, contained little vitamin C. Little was known concerning an antirachitic factor, distinct from vitamin A, at this time.

CONDUCT OF EXPERIMENT

The experiment was carried out during the winter months. The dairy products for the ice cream mixes were from cows which were in the barn most of the time and were obtained at a time of the year when the ultraviolet intensity of the sun's rays was low. Standard ice cream mixes were used, having been previously processed according to commercial practices. The only differ-

---

* Received for publication November 26, 1928. The material presented in this paper is a part of a thesis submitted by Mr. Orme J. Kahlenberg in partial fulfillment of the requirements for the degree of Master of Science, Rutgers University. The preparation of this paper is largely the work of Mr. Kahlenberg.

ence in the product was the irradiation given the mix before use in the feeding trials.

TABLE 1

The effect on rachitic rats of the feeding of irradiated and non-irradiated ice cream containing 8 per cent fat

TREATMENT OF ICE CREAM MIX AND PERCENTAGE FED (MIX CONTAINED 8 PER CENT FAT)	RAT NUMBER	SEX	GRADE OF TEST	GAIN IN WEIGHT, 5-DAY TEST PERIOD	AVERAGE DAILY FOOD CONSUMPTION	PERCENTAGE BONE ASH OR INORGANIC BLOOD PHOSPHORUS PER 100 CC. BLOOD
				grams	*grams*	
Four per cent fed. Irradiated 10 minutes at room temperature	627	M.	+++	59–63	5.2	Bone ash, 28.6 per cent
	628	F.	++	60–62	6.0	Bone ash, 27.0 per cent
	665	M.	++	76–84	9.2	Blood phosphorus, 2.74 mgm.
	671	M.	+	73–82	8.8	
	672	F.	++	60–66	7.0	
	667	M.	+	70–76	8.4	Blood phosphorus, 2.05 mgm.
	673	F.	++	63–72	9.0	
	676	F.	+	67–74	8.4	
	678	F.	+++	63–70	8.2	
Eight per cent fed. Irradiated 10 minutes at room temperature	629	F.	++++	64–65	6.6	Bone ash, 26.4 per cent
	630	F.	+	59–62	5.6	Bone ash, 26.8 per cent
	606	M.	++++	60–64	6.6	Bone ash, 23.6 per cent
	607	F.	++++	57–61	6.8	Bone ash, 23.0 per cent
Eight per cent fed. Non-irradiated mix	592	M.	–	65–69	6.0	Ash, 20.8 per cent
	594	M.	–	72–78	8.6	Ash, 22.4 per cent
	931	M.	–	65–73	7.0	Blood phosphorus, 1.87 mgm.
	932	F.	–	49–55	6.2	
	933	F.	–	50–57	6.4	
	934	M.	–	61–69	6.6	
Control. At beginning of test period	601	M.	–	63		Bone ash, 24.3 per cent
	608	F.	–	61		Bone ash, 26.8 per cent
Control. At end of test period	634	M.	–	59–60	5.4	Bone ash, 27.9 per cent
	639	F.	–	56–60	5.0	Bone ash, 22.1 per cent
	658	F.	–	66–70	6.6	
	687	M.	–	94–101	8.8	Blood phosphorus, 2.05 mgm.
	691	M.	–	79–87	8.6	

Biological technique

The biological technique used is similar to that described by Bills, Honeywell and MacNair (4) but with the following differ-

TABLE 2

The effect on rachitic rats of the feeding of irradiated and non-irradiated ice cream containing 12 per cent fat

Influence of temperature of irradiation

TREATMENT OF ICE CREAM MIX AND PERCENTAGE FED (MIX CONTAINED 12 PER CENT FAT)	RAT NUMBER	SEX	GRADE OF TEST	GAIN IN WEIGHT, 5-DAY TEST PERIOD	AVERAGE DAILY FOOD CONSUMPTION	INORGANIC BLOOD PHOSPHORUS PER 100 CC. BLOOD
				grams	*grams*	*mgm.*
Six per cent fed. Irradiated 2 minutes. At 15.5°–43.0°C. (60°–110°F.)	679	M.	++	69–73	7.4	4.22
	681	F.	++	65–70	7.0	
	692	F.	+	54–58	6.2	
At 43.0°–62.5°C. (110°–145°F.)	680	F.	+	67–71	6.8	4.53
	689	M.	++	86–93	8.8	
	690	M.	++	75–86	9.4	
Four per cent fed. Irradiated 2 minutes. At 15.5°–43.0°C. (60°–110°F.)	839	M.	−	74–77	7.2	2.15
	840	F.	−	75–80	7.8	
	841	M.	−	75–78	7.2	2.13
	842	F.	−	72–75	7.2	2.23
	843	M.	+	73–77	7.0	2.06
At 43.0°–62.5°C. (110°–145°F.)	715	M.	+	62–68	7.0	2.37
	716	F.	+	63–65	6.8	
	720	F.	−	78–84	6.6	
Six per cent non-irradiated	683	F.	−	64–67	7.0	1.38
	684	M.	−	59–62	6.4	
	685	M.	−	71–77	7.8	
	928	M.	−	74–83	8.0	1.54
	929	M.	−	65–73	7.6	
	930	F.	−	61–66	6.6	
Control. Beginning of test period	799	F.	−	67		1.92
	800	M.	−	61		
	801	F.	−	50		
	802	M.	−	66		
	803	M.	−	73		
Control. End of test period	794	M.	−	61–68	6.4	
	795	M.	−	56–59	7.2	
	796	F.	−	60–68	9.4	
	797	F.	−	68–75	9.2	
	798	M.	−	68–72	7.6	

ences. The stock ration was Sherman No. 13, consisting of two-third whole wheat, one-third whole milk powder, and 2 per cent of the weight of the wheat as sodium chloride (5), modified by the addition of 5 grams of raw beef per day or a meat scrap incorporated in the ration to the extent of 10 per cent, at the proportional expense of the wheat and milk. The rats were not kept in a dark room. The experimental diet for the production of rickets was Steenbock's No. 2965, a high calcium low phosphorus ration (6). Invariably the individuals examined had developed rickets at the end of twenty days. That the ration and the experimental conditions produced and maintained the rachitic condition throughout the five-day test period is demonstrated by the records of control animals in tables 1 and 2. Although it is not a part of the usual procedure, the rats were weighed daily during the five-day period to determine whether or not those showing a steady and uniform gain had better bone calcification, as judged by the Shipley line-test, than those that showed gains and losses. Any differences were not marked enough to be detected by the Shipley technique.

The degree of healing was judged primarily by the Shipley line test (7) but comparisons were made, on some of the individuals, of the line test with the bone ash percentage of the dry, extracted femurs (8) and on others with the inorganic phosphorus of the blood. The ash of each femur was determined and the results averaged. The degree of calcification was determined by a published, arbitrary scale of plus signs (9).

+ Healing rickets, indicating a just perceptible healing.
++ Healing rickets, indicating a distinct healing.
+++ Healing rickets, indicating advanced healing.
++++ Healing rickets, indicating practically a complete healing, the amount of calcium being normal and only some structural evidence of rickets remaining.

To the above scale the following signs were added in this investigation.

−? Doubtful healing.
− Severe rickets or no healing.

In this experiment a two-plus healing was desired as a cure. In order to get the maximum amount of blood in the very young rats, it was obtained from the anesthetized animal by cutting through the thorax and drawing the blood directly from the heart, by a 2 cc. Luer syringe, using needles 21 to 23 bore and $\frac{1}{2}$ to $\frac{5}{8}$ inches long. The inorganic phosphate content of the blood was determined by the Briggs modification (10) of the Bell-Doisy method. Citrated plasma was used when a pooled sample was obtained but oxalated whole blood was employed when only individual samples were available.

Preparation of ice cream

The ice cream mixes were pasteurized at 62.5°C. (145°F.) for thirty minutes, viscolized at a pressure of 2500 pounds, then cooled to the aging temperature 1°C. (34°F.), and allowed to age for twenty-four hours. Some mixes were irradiated at the desired temperature before freezing and others afterward, but this difference in procedure had no effect on the antirachitic property. They were then stored in brown, stoppered bottles at the hardening room temperature −23°C. (−9.4°F.)

For the irradiation 25 to 50 cc. were exposed in a thin film in a shallow Pyrex pie dish, 9 inches in diameter, to the radiant energy generated by the quartz mercury burner of a Cooper Hewitt Uviarc Poultry Treater lamp, at a distance of 12 inches for the two- and ten-minute exposures. The exposures were begun at room temperature, unless otherwise stated, a slight rise being observed as the irradiation progressed. Later, the distance was decreased to 4 inches and the time to 15 seconds. The burner was operated at 110 volts, A.C., (D.C. equivalent 63 volts). The arc wattage was 270 watts. The literature had warned against the fishy flavor of irradiated dairy products, due possibly to the formation of ozone (11). An attempt was made to drive off any ozone by passing an air current from an electric fan between the mix and the lamp during irradiation. With ten-minute exposures this precaution did not seem to have any effect of avoiding the fishy flavor. The flavor with the two-minute and fifteen-second exposures was not objectionable even without the fan.

EXPERIMENTAL RESULTS

Two groups of rats were fed ice cream mixes, which differed with respect to the percentage of fat. Group I received a mix consisting of 8 per cent fat, 11 per cent serum solids (solids-not-fat), 15 per cent sugar and 0.3 per cent gelatin. Group II received a 12 per cent fat ice cream, a standard used by many of the leading ice cream manufacturers in this country. The mix was standardized to 12 per cent fat, 11 per cent serum solids (solids-not-fat), 15 per cent sugar and 0.3 per cent gelatin.

Irradiation experiments

After preliminary trials in which various periods of irradiation were used and various percentages of the mix were incorporated in the ration, it was found that the desired two-plus healing could be accomplished when an 8 per cent fat ice cream, irradiated for 10 minutes, was fed at a 4 per cent level. Table 1 presents the rat records obtained when 4 per cent of this mix was incorporated in the ration. The results of feeding an 8 per cent level are included to demonstrate the effect of a higher intake. That the non-irradiated ice cream did not cure the rachitic condition is amply demonstrated. It is possible, however, that a cure could be obtained with a non-irradiated product if a large enough dose were fed.

It is observed from the results in table 2 that with an increase in butterfat the exposure time could be reduced to two minutes. To obtain the desired two-plus healing, however, the level of feeding had to be increased to 6 per cent of the diet. The fact that fat globules clumped together, giving the appearance of particles of butter, led to the idea of heating the mix so that one would get a more fluid condition. By doing this it was thought that more surface would be exposed and hence greater activation attained. As the temperature at which the ice cream mix leaves the viscolizer in a commercial plant is 62.5°C. (145°F.), and a little below, some of the mixes were heated to this temperature. From the results presented in table 2, apparently the difference in the temperature at which the ice cream mixes were irradiated has no effect on the antirachitic potency of the product. The problem of the difference in potency for long periods of storage of mixes irradiated at different temperatures was left for future investigation. The non-irradiated 12 per cent fat ice cream from the same batch failed to heal rickets when the rats were given a dosage equal to the maximum amount of the irradiated product fed.

TABLE 3

The curative effect of ice cream irradiated for 15 seconds at 4 inches

TREATMENT OF ICE CREAM MIX AND PERCENTAGE FED (MIX CONTAINED 12 PER CENT FAT)	RAT NUMBER	SEX	GRADE OF TEST	GAIN IN WEIGHT, 5-DAY TEST PERIOD	AVERAGE DAILY FOOD CONSUMPTION	INORGANIC BLOOD PHOSPHORUS PER 100 CC. BLOOD
				grams	*grams*	*mgm.*
Four per cent fed. Irradiated 15 seconds at 4 inches. 34° to 37°C. (93° to 98°F.)	832	M.	++	68–73	9.0	6.73
	833	M.	++	60–65	7.4	
	834	F.	−?	55–57	6.2	
	835	M.	++	72–75	8.2	
	836	F.	+	60–63	6.6	

TABLE 4

The effect of storage on irradiated 8 per cent fat ice cream

TREATMENT OF ICE CREAM MIX AND PERCENTAGE FED	RAT NUMBER	SEX	GRADE OF TEST	GAIN IN WEIGHT, 5-DAY TEST PERIOD	AVERAGE DAILY FOOD CONSUMPTION	INORGANIC BLOOD PHOSPHORUS PER 100 CC. BLOOD
				grams	*grams*	*mgm.*
Irradiated 10 minutes, November 26, 1927. Tested, January 26, 1928. Fed at 4 per cent level	666	F.	++	64–72	8 6	
	668	M.	++	76–86	9.4	2.41
	669	M.	++	73–81	9.4	
	670	F.	+++	67–74	9.0	
	674	M.	++	76–87	9.8	
	675	M.	++	66–74	8.6	
	677	F.	++	66–68	8.2	

The fact that the intensity of light is inversely proportional to the square of the distance from the source, led to the assumption that one could probably get the same degree of healing when the distance was shortened to 4 inches and a 15-second exposure period used. Table 3 shows that a healing was obtained with this distance and with such a short exposure. The temperature of the irradiated mix rose from 34° to 37°C. (93° to 98°F.) during the fifteen seconds of irradiation, a difference that would have very little effect on the antirachitic properties of the product, according to table 2.

Storage of irradiated ice cream

It is observed in table 4 that the desired two plus healing was obtained after two months of storage at −23°C. (−9.4°F.). The records of the animals on this test are to be compared with those of Rats 627 and 628 in table 1, which were fed the same irradiated mix two months previously, and with those of rats in table 1, which received other samples of 10 minute-irradiated, 8 per cent mix at the 4 per cent level.

TABLE 5

Comparison of the Shipley line rating and the percentage of bone ash

	SHIPLEY LINE RATING				
	−	+	++	+++	++++
	Beginning of test period				
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Percentage of bone ash. average of the two femurs	24.3	19.4	19.5	19.9	23.0
	26.8	20.9	22.0	20.1	24.0
	27.6	21.0	24.6	23.6	26.4
	32.0	23.2	24.7	25.0	23.6
	After 5-day test period	26.8	27.0	28.6	
		27.1		30.9	
		27.3			
	20.8	29.5			
	22.1				
	22.3				
	22.3				
	22.4				
	27.9				

Comparison of the line test with the bone ash percentage

In table 5 are shown the bone ash percentages and the corresponding rating by the Shipley line technique of some of the animals whose records appear in the preceding tables and of animals used in preliminary studies, but whose records are not reported. The ash of the rachitic rat femurs varied from 20.8 to 32.0 per cent and that of the healed rats to about the same extent. Hence, there is no correlation between the degree of healing and the percentage of bone ash. This finding is in agreement with the results reported recently by Adams and McCollum (12). It is suggested that, in as short a period as five days, not enough additional calcium and phosphorus are deposited in the bones to be detected by the method employed for the determination of bone ash.

TABLE 6

Comparison of the line test and inorganic blood phosphorus values

LINE TEST	ESTIMATED GROUP AVERAGE	INORGANIC PHOSPHORUS PER 100 CC BLOOD
		mgm.
— Average of 31 animals minimum value 1.21 maximum value 2.23	—	1.76
1+	1+	2.05
1+	1+	2.06
1+, 1+, 1+,	1+	3.04
2+	2+	2.13
2+	2+	2.41
2+, 2+,	2+	2.39
1+, 2+, 2+,	2+	2.74
1+, 2+, 2+,	2+	4.22
1+, 2+, 2+,	2+	4.53
– ?, 1+, 2+, 2+, 2+,	2+	6.37
2+, 2+, 3+,	2+	2.89
2+, 2+, 3+,	2+	2.41
2+, 3+, 3+,	3+	3.78
2+, 4+,	3+	4.28

Comparison of the line test readings with the inorganic blood phosphorus

Because of the fact that pooled samples were used for the phosphate determination in almost every instance, a direct comparison of these results with the individual results of the line test cannot be made.

Reference to table 6 reveals an increase in the phosphate level when a healing has taken place, a fact generally recognized, but the amount of the increase is not always in agreement with the extent of the cure. Occasionally a rachitic animal will show a higher phosphate level than one in which healing has taken place. The values shown are not quite as comparable as they would be if a substance were being tested which is ingested in small amounts, such as cod-liver oil or irradiated ergosterol, because the mineral content of the larger doses of ice cream might disturb the mineral balance of the ration. Although an increase in the phosphate level generally accompanies a healing of the rachitic condition, there is only general accord between the two observations. These results are in agreement with those of the more extensive inquiry of Adams and McCollum (12).

SUMMARY

These observations serve primarily as an additional item to the evidence which is being accumulated concerning vitamins and the food value of ice cream.

1. From the data presented in this paper it is concluded that an irradiated ice cream mix, prepared from winter cream, heals rickets in the white rat, whereas the non-irradiated product of the same batch of ice cream, fed at a maximum level of 8 per cent of the ration, does not produce a healing.
2. A variation of the temperature of the product during irradiation between 15.5° and 62.5°C. (60° to 145°F.), does not affect the activity of the material.
3. Freezing and low storage temperature do not affect the antirachitic value of irradiated ice cream for at least two months.
4. A definite correlation was not found between the inorganic blood phosphorus level and the degrees of healing as expressed in using the Shipley line technique, there being only a general accord between the two observations. There is no agreement between the results of the Shipley line test and the percentage of bone ash.

REFERENCES

(1) Hess, A. F. 1924 Proceedings of the American Pediatric Society, June, 1924. Abstracted in Amer. Jour Dis. Children, xxviii, 256.

(2) Steenbock, H., and Black, A. 1924 The induction of growth-promoting and calcifying properties in a ration by exposure to light Science, lx, 224; Fat soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultraviolet light. Jour. Biol. Chem., lxi, 405.

(3) Smith, A. H. 1922 Vitamins in ice cream. Jour. Amer. Med. Assoc., lxxix, 2221.

(4) Bills, C. E., Honeywell, E. M., and MacNair, W. A. 1928 Antiricketic substances. VII. Biochemical and spectroscopic studies on purified cholesterol. Jour. Biol. Chem., lxxvi, 251.

(5) Sherman, H. C., and Campbell, H. L. 1924 Growth and reproduction upon simplified food supply. IV. Improvement in nutrition resulting from an increased proportion of milk in the diet. Jour. Biol. Chem., lx, 5.

(6) Steenbock, H., and Black, A. 1925 Fat-soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. Jour. Biol. Chem., lxiv, 263.

(7) McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A. 1922 Studies on experimental rickets. XVI. A delicate biological test for calcium-depositing substances. Jour. Chem., li, 41.

(8) Bethke, R. M., Steenbock, H., Nelson, M. T. 1923 Fat-soluble vitamins. XV. Calcium and phosphorus relations to growth and composition of blood and bone with varying vitamin intake. Jour. Biol. Chem., lviii, 71.

(9) Bills, C. E., and McDonald, F. G. 1926 Antiricketic substances. IV. The polymerization of cholesterol. Jour. Biol. Chem., lxviii, 821.

(10) Briggs, A. P. 1922 A modification of the Bell-Doisy phosphate method. Jour. Biol. Chem., liii, 13.

Briggs, A. P. 1924 Some applications of the colorimetric phosphate method. Jour. Biol. Chem., lix, 255.

(11) Spinka, J. 1924 Untersuchungen über den Einfluss ultravioletter Strahlen auf akzessorische Stoffe. I. Versuche uber die Beeinflussung des Faktors A. Biochem. Z., cliii, 197.

(12) Adams, G., and McCollum, E. V. 1928 A method for the biological assay of codliver oil. Jour. Biol. Chem., lxxviii, 495.

STUDIES ON THE NUTRITIVE VALUE OF MILK

II. THE SUPPLEMENTARY VALUE OF INORGANIC IRON AND COPPER*

W. E. KRAUSS

Ohio Agricultural Experiment Station, Wooster

In a previous paper (1) it was shown that anemia was produced in weanling rats from normal mothers in from 4 to 6 weeks, by feeding an exclusive whole milk diet. Since it was desired to use the exclusive milk method of feeding for comparing the value of milk from different sources, it became necessary to search for a supplement that would prevent the development of anemia. Such a supplement would need to be sufficiently potent to insure protection through the addition of a very small quantity, particularly if a food substance. If a chemical, it could not be of such a nature as to bring about a reaction that would alter the original form of the milk.

For a long time anemia has been attributed to a shortage of iron. This is probably due to the fact that iron is found in the hemoglobin of most species of animals and that when hemoglobin is broken down it yields, among other things, an iron-containing radicle. Also, iron is a good carrier of oxygen, and one of the important functions of hemoglobin is to carry oxygen to various parts of the body. It was natural, then, to turn first of all to some source of iron as the necessary supplement. Inorganic sources of iron were chosen because of the work of Hart, Elvehjem and Herrin (2), which showed that the ash of lettuce or cabbage corrected anemia, and of Mitchell and Schmidt (3) which showed considerable success through the use of certain inorganic iron salts. More recent work by Waddell, Elvehjem, Steenbock and Hart (4) has confirmed the belief that the antianemic factor is inorganic in nature, and further, that copper is essential as a supplement to iron for hemoglobin building in the rat (5). A

---

* Received for publication November 27, 1928.

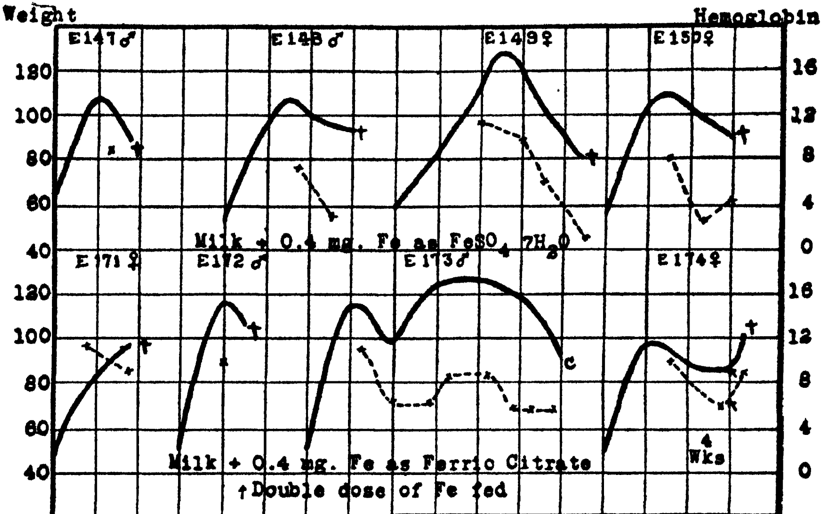
preliminary report by McHargue, Healy and Hill (6) has also pointed out the importance of copper in hemoglobin formation in the rat. The copper additions in the experiments here reported were made after the work of Hart and associates (5) was made public.

The technique employed was essentially the same as that already described (1). However, instead of applying silver nitrate and alum powder to the tail after cutting, a hot spatula was applied momentarily. This proved to be much more satisfactory as it reduced the loss of blood to a minimum. While it was found by Elvehjem, Herrin and Hart (7) that the cow's feed did not influence the iron content of the milk, the precaution was taken to use milk from the same cows throughout. Milk from three cows fed a normal ration and not given access to pasture was saved each day and combined for feeding. Samples of milk from each cow were analyzed for iron from time to time by the method of Elvehjem and Hart (8). While the iron content of the milk from each cow ranged from 0.00017 to 0.00035 per cent, the average iron content of the combined samples approached very closely that reported by Hart and Elvehjem, viz., 0.00024 per cent.

As an iron supplement, ferrous sulfate (Baker's C.P.) was first tried. A solution of the salt was made so that 1 cc. of the solution contained 0.4 mgm. of iron. This quantity of iron was considered by Abderhalden (9) to be sufficient for the requirements of the rat. Mitchell and Schmidt (3) found that when 0.4 mgm. of iron from an easily available source was added to the diet of anemic rats a rapid rise in hemoglobin occurred. Consequently, 1 cc. of iron solution was incorporated in the milk of each rat. The iron addition was made as soon as the rats were placed on experiment. It was added to a sufficiently small amount of milk in the afternoon to insure complete consumption. Additional milk was then fed in the morning.

The growth curves of the rats receiving the ferrous sulfate addition, together with the trend of the hemoglobin content of the blood, are given in figure 1. Rat E 147 developed severe respiratory trouble shortly after the first hemoglobin determina-

FIG. 1



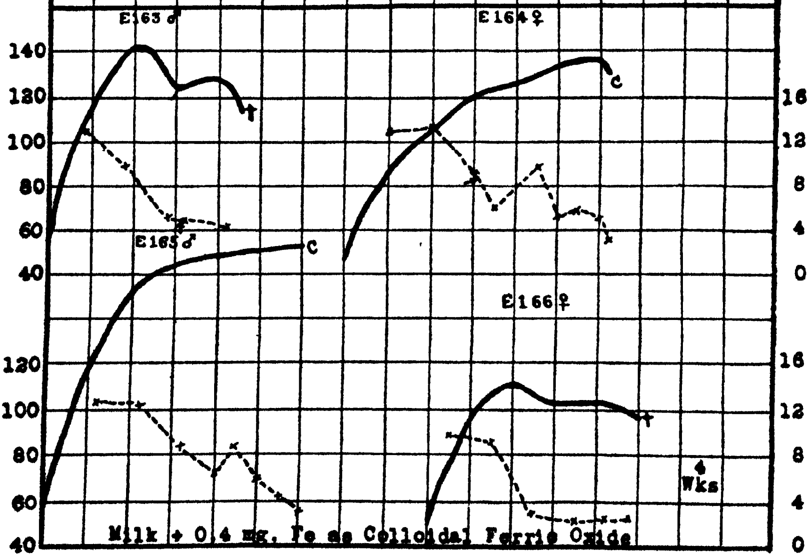


FIG. 2

FIG. 1. This chart shows that the addition of either ferrous sulfate or ferric citrate did not prevent anemia from developing.

In this and succeeding charts, growth is indicated by the solid lines, and hemoglobin (grams per 100 cc. of blood) by the broken lines.

FIG. 2. Evidently the colloidal nature of the iron fed to this group had no effect on the development of anemia. However, all the rats in this group survived longer than controls getting no iron supplement, and those getting ferrous sulfate or ferric citrate.

tion was made and was in such poor condition that no further data as to hemoglobin were obtained. The only effect of the ferrous sulfate was to reduce slightly the rate at which the hemoglobin content of the blood decreased. It may be of significance that one rat in this group died of respiratory trouble and another, E 149, had puffy eyes during the last few weeks of the trial.

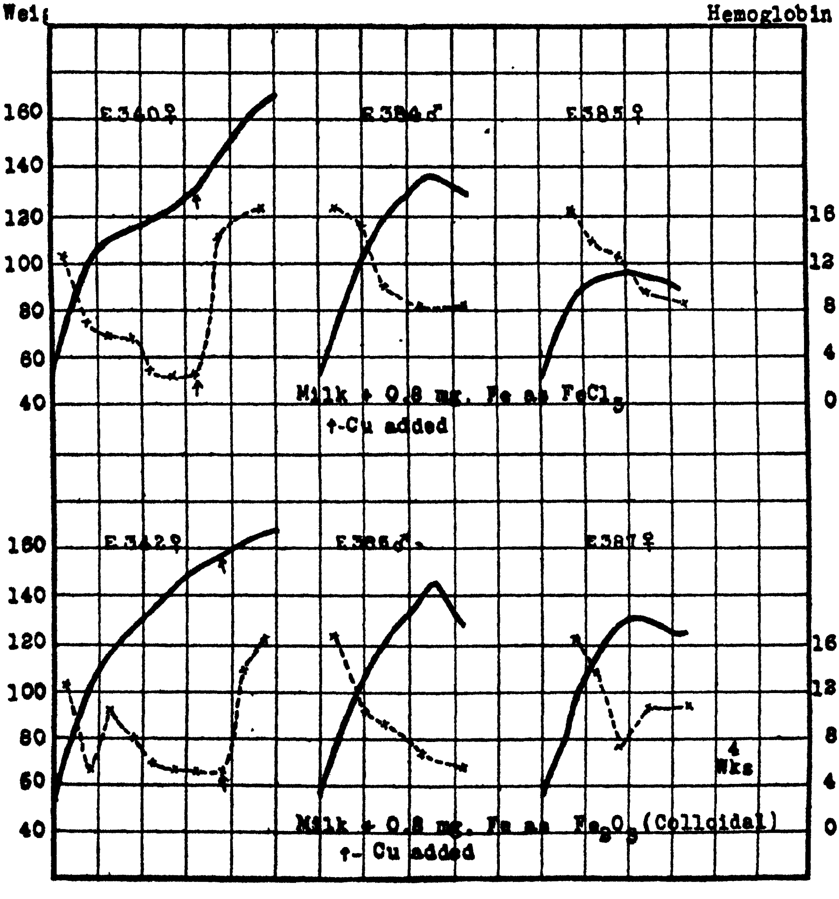


FIG. 3. This chart compares the antianemic potency of ferric chloride and colloidal ferric oxide made from ferric chloride. No difference in the efficiency of these two forms of iron is apparent. The remarkable effect of the addition of copper as copper sulfate is shown by the hemoglobin curves of Rats E 340 and E 342.

Similar results were obtained with ferric citrate (Baker's C.P.) figure 1. Respiratory trouble and eye trouble prevailed in this group. The conjunctiva became red and swollen and the lids were sticky. This condition closely resembled xerophthalmia due to a vitamin-A deficiency. It might appear that both ferrous sulfate and ferric citrate tend to induce the "salt ophthalmia" described by McCollum, Simmonds, and Becker (10) (11), and explained by Jones (12) as due to destruction of vitamin A through oxidation. Our results are not in accord with those of McCollum and associates, who found that when ferrous sulfate was replaced by ferric citrate the ophthalmia disappeared. Furthermore, ulceration and perforation of the eyeballs has been observed in this laboratory in rats on an exclusive milk diet, with no iron addition.

Thinking that the state of the iron might influence its availability, a colloidal solution of ferric oxide was prepared and fed at the same level and in the same manner as the other iron additions. The results (fig. 2), show that the colloidal nature of the iron did not enable it to prevent anemia from developing. However, life was prolonged considerably and the rats were in better condition than those receiving either ferrous sulfate or ferric citrate.

As a further check on this point a colloidal solution of ferric oxide was prepared from ferric chloride and fed to one group of rats. Another group of rats was fed a true ferric chloride solution of equal iron content. In order to insure an adequate iron intake, 0.8 mgm. iron, twice the amount found by others to meet the requirements, was fed to each rat. This trial showed no advantage in colloidal ferric oxide over ferric chloride (fig. 3). It demonstrated also that even with such a high iron intake the hemoglobin content of the blood decreased rather rapidly. This indicated that some factor other than iron was necessary for hemoglobin formation in the rat.

About this time the information contained in the article by Hart and associates (5), was made public. Consequently copper sulfate was tried, together with iron, to overcome the anemia with which we were concerned. The results of these trials were so

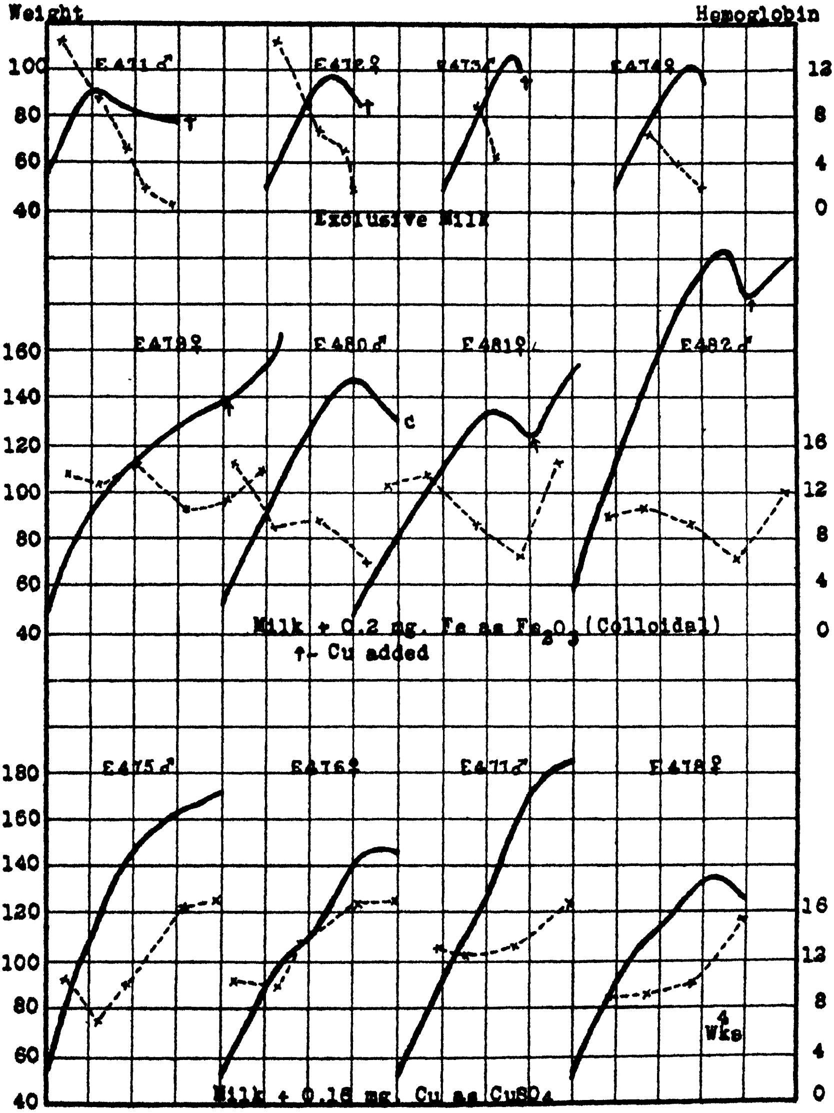


FIG. 4. This shows the course of growth and hemoglobin picture on an exclusive milk diet, the effect of the addition of colloidal iron, and the effect of copper alone. The lowest set of curves show that the addition of 0.16 mgm. of copper as copper sulfate, without any iron supplement, was sufficient to prolong life and bring the hemoglobin up to a high level. A definite explanation of this cannot be made.

striking that some of them are reported here as confirmatory evidence.

Figure 4 shows the effect of adding iron or copper to an exclusive milk diet. A control group is shown for comparison. Two-tenths milligram iron as colloidal ferric oxide was added to the milk fed one group, and 0.16 mgm. copper as copper sulfate to the milk of the other group. The addition of iron allowed good growth for three months, but did not prevent the hemoglobin content of the blood from decreasing. After growth had ceased and hemoglobin was at a low level, 0.16 mgm. of copper as copper sulfate was added. This resulted in an immediate response, both in increased weight and amount of hemoglobin. (Rat E 480 was killed because of a tail infection).

The addition of copper alone, on the other hand, not only allowed good growth, but caused the hemoglobin content of the blood to slowly rise to the normal level. This can not be explained on the basis of the iron impurity present in C.P. anhydrous copper sulfate, as the amount of iron as impurity in the copper sulfate furnished each rat daily amounted to only 0.000008 mgm. Since the amount of iron furnished by the milk amounted to only 0.10 to 0.20 mgm. it appears that either there is a requirement for a very small amount of copper for hemoglobin formation, or else the iron present in the milk is made more available through the presence of copper. If the second view is correct, it follows that the iron requirement of the rat is not as great as formerly supposed. If the copper exerts only a catalytic effect the amount present in the milk consumed daily, amounting to 0.025 mgm. (based on an average consumption of 50 cc. of milk containing, according to our analysis by the Biazzo method (13), 0.5 mgm. copper per liter) should exert some effect on the availability of the iron in the milk. That more copper than that present in the milk is necessary indicates that copper might perform some function other than one catalytic in nature.

The effect of adding copper to the milk and iron diet of anemic rats is strikingly shown in the curves for rats E481 and E482 (fig. 4) and rats E340 and E342 (fig. 3). This confirms the historic case described by Hart and associates, and has been observed repeatedly in this laboratory without a record of a single failure.

Figure 5 offers further evidence of the supplementary effect of copper and iron additions to milk, and shows that the iron requirement of the rat is certainly no greater than 0.4 mgm. Just as good results were obtained with 0.2 mgm. as with 0.4 mgm. additions of iron.

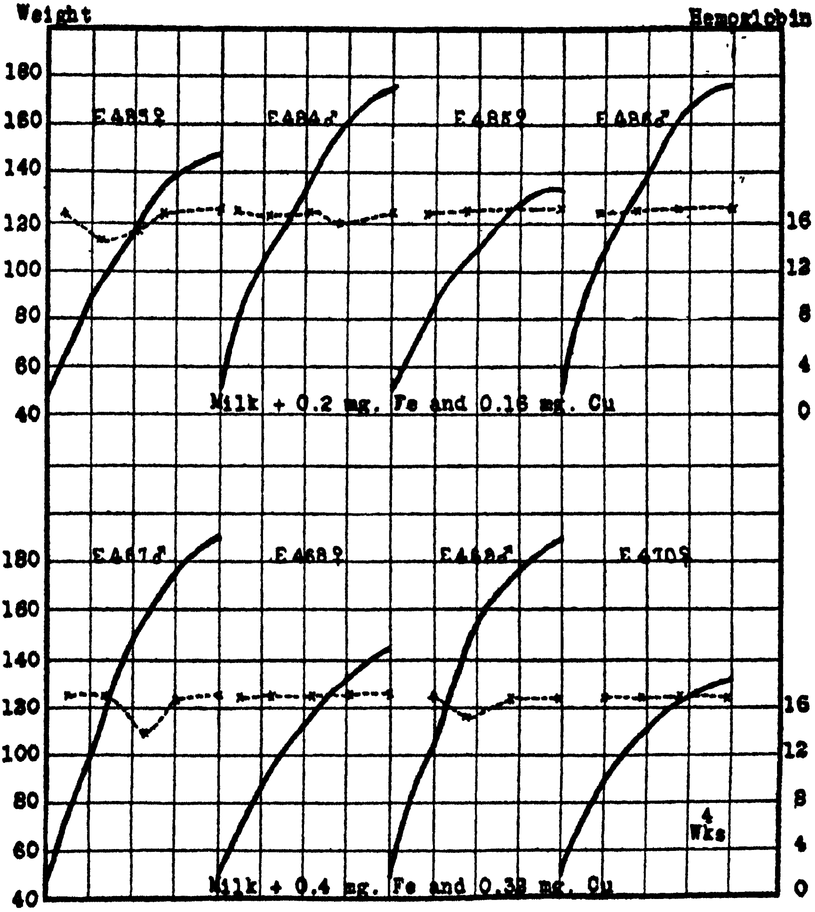


Fig. 5. That the addition of 0.2 mgm. Fe rather than the 0.4 mgm. usually recommended will allow excellent growth and hemoglobin formation when supplemented with copper is shown in this chart.

DISCUSSION

While it has long been recognized that the most serious dietary deficiency of milk is its low iron content, it would seem that there is an equally serious deficiency of copper. It is also apparent that for hemoglobin formation in the rat the availability of the iron is more important than the amount present and that the iron is made more available through the addition of copper.

Through the addition of a small amount of iron and a small amount of copper, it should be possible to use the exclusive whole-milk diet of feeding for determining the total nutritive effect (aside from reproduction) of milk produced under different conditions. The growth curves as shown in figure 5 are below normal compared to those of our stock rats, but the food intake was limited. In spite of the fact that we have kept males and females together for long periods on such a supplemented milk diet, no offspring have ever been produced.[1]

SUMMARY

Ferrous sulfate, ferric citrate, ferric chloride and colloidal ferric oxide were found ineffective in preventing anemia in rats when added to an exclusive whole milk diet. The addition of a small amount of copper (0.16 mgm. daily) as copper sulfate was quite effective. The addition of both copper and iron proved highly effective in preventing nutritional anemia in rats. This confirms the work of Hart, Steenbock, Waddell, and Elvehjem (5).

The prevention of nutritional anemia in rats fed an exclusive milk diet through the addition of small amounts of copper and iron suggests a method for determining the total nutritive effect (aside from reproduction) of milk produced under various conditions, without the use of a basal ration.

---

[1] Since writing the manuscript for this article, Waddell and Steenbock (Jour. Biol. Chem., lxxx, 431–42, 1928) have shown that the vitamin-E potency of a ration may be destroyed by treatment with ferric chloride. This may explain the reproductive failures encountered here.

REFERENCES

(1) KRAUSS, W. E. 1929. Studies on the nutritive value of milk. I. The production of nutritional anemia in albino rats through exclusive whole milk diets. Jour. Dairy Sci, xii, 74–79.

(2) HART, E. B., ELVEHJEM, C. A., AND HERRIN, R. C. 1927 Iron in nutrition. IV. Nutritional anemia on whole milk diets and its correction with the ash of certain plant and animal tissues or with soluble iron salts. Jour. Biol. Chem., lxxii, 299–320.

(3) MITCHELL, HELEN S., AND SCHMIDT, LOLA. 1926 The relation of iron from various sources to nutritional anemia. Jour. Biol. Chem., lxx, 471–486.

(4) WADDELL, J., ELVEHJEM, C. A., STEENBOCK, H., AND HART, E. B. 1928 Iron in nutrition. VI. Iron salts and iron-containing ash extracts in the correction of anemia. Jour. Biol. Chem., lxxvii, 777–795.

(5) HART, E. B., STEENBOCK, H., WADDELL, J., AND ELVEHJEM, C. A. 1928 Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. Jour. Biol. Chem., lxxvii, 797–812.

(6) McHARGUE, J. S., HEALY, D. J., AND HILL, E. S. 1928 The relation of copper to the hemoglobin content of rat blood. Preliminary report. Jour. Biol. Chem., lxxviii, 637–641.

(7) ELVEHJEM, C. A., HERRIN, R. C., AND HART, E. B. 1927 Iron in nutrition. III. The effect of diet on the iron content of milk. Jour. Biol. Chem., lxxi, 255–262.

(8) ELVEHJEM, C. A., AND HART, E. B. 1926 Iron in nutrition. II. Quantitative methods for the determination of iron in biological materials. Jour. Biol. Chem., lxvii, 43–51.

(9) ABDERHALDEN, E. 1900 Die resorption des Eisens, sein Verhalten im Organismus und seine ausscheidung. Z. Biol., xxxix, 113–252.

(10) McCOLLUM, E. V., SIMMONDS, NINA, AND BECKER, J. E. 1922 On a type of ophthalmia caused by unsatisfactory relations in the inorganic portion of the diet. An ophthalmia not due to starvation for fat-soluble A and not curable by its administration. Jour. Biol. Chem., liii, 313–321.

(11) McCOLLUM, E. V., SIMMONDS, NINA, AND BECKER, J. E. 1925 Further studies on the cause of ophthalmia in rats produced with diets containing vitamin A. Jour. Biol. Chem., lxiv, 161–179.

(12) JONES, J. H. 1927 The relation of the inorganic constituents of a ration to the production of ophthalmia in rats. Jour. Biol. Chem., lxxv, 139–146.

(13) BIAZZO, R., 1926 Metodo rapido di determinazione del rame nelle conserve di prodotti vegetali rinverditi. Annali di Chimica Applicata, vol. 16, Fasc. 2.

METALS IN DAIRY EQUIPMENT

CORROSION CAUSED BY WASHING POWDERS, CHEMICAL STERILIZERS, AND REFRIGERATING BRINES*

O. F. HUNZIKER, W. A. CORDES, AND B. H. NISSEN

Blue Valley Creamery Company, Research Laboratories, Chicago, Illinois

In a previous paper entitled "Metals in Dairy Equipment," which appeared in the March, 1928, issue of THIS JOURNAL (1), the results were given of an experimental study of the effect of nineteen different metals, plated metals, and metallic alloys, such as may be used in the manufacture of dairy equipment, on the flavor of milk and milk products and the effect of the dairy product on the various metals themselves.

In the present experiment these same metals were exposed to various dairy cleansers, to chemical sterilizers, to neutralizing lime, to refrigerating brines, and to successive treatment with steam, cooling water, and brine.

The importance of resistance to corrosion of metallic dairy equipment in alkaline washing powders, neutralizers, and chemical sterilizers, is self-evident. The cleaning of such equipment makes indispensable the use of alkalies, and yet the action of these alkalies on the metals must inevitably influence the resistance to and effect of the metals on the milk product. The life of the equipment is an additional important consideration and it is to a large extent dependent on the resistance of the metals to steam treatment and refrigerating brines.

EXPERIMENTAL

Materials used. The laboratory tests included 19 different metals, plated metals, and metallic alloys. These metals were used in the form of strips 4 inches long and 1⅜ inches wide, and were as follows:

---

* Received for publication December 11, 1928.

Single metals

- Aluminum[*]
- Copper
- Iron
- Nickel
- Tin
- Zinc

Plated metals

- Galvanized iron
- Tinned copper
  - Edges bare[§]
  - Edges tinned[**]
- Copper Retin[‡]
- Tinned iron
  - Edges bare[††]
  - Edges tinned[‡‡]
- Manganese aluminum alloy (aluminum plated)[*]

Metallic Alloys

- Allegheny Metal[†]
- Ascoloy[†]
- Enduro Type "A"[§§]
- Manganese aluminum alloy[*]
- Monel Metal
- Nickel silver

[*] Furnished by the Aluminum Company of America. The aluminum is the pure metal in a soft condition. The manganese aluminum alloy consists of 1.25 per cent manganese and the balance aluminum.

[†] Furnished by the Allegheny Steel Company. Ascoloy is an ordinary chromium steel alloy, containing approximately 12 to 16 per cent Cr, 0.5 per cent Si, 0.5 per cent Mn, 0.025 per cent P, and 0.025 per cent S. Allegheny Metal is a chromium-nickel steel alloy, containing 17 to 20 per cent Cr, 7 to 10 per cent Ni, and less than 0.5 per cent Mn, 0.5 per cent Si, 0.025 per cent P, and 0.025 per cent S.

[‡] Furnished by the Sorensen Specialty Company. This is copper coated with a tin preparation which can be applied to renew the tinned surface without use of flux. The tin coating was very heavy.

The remaining metals were furnished by J. G. Cherry Company.

[§] Represents copper strips the cut edges of which were not tinned.

[**] Represents copper strips the cut edges of which were tinned.

[††] Represents iron strips the cut edges of which were not tinned.

[‡‡] Represents iron strips the cut edges of which were tinned.

[§§] Enduro Type "A" is an ordinary or straight chromium steel containing 16.5 to 18.5 per cent Cr, 0.5 to 1.25 per cent Si; and less than 0.1 per cent C, 0.5 per cent Mn, 0.03 per cent S, 0.03 per cent P, and 0.25 per cent Ni. Since completing this work our attention was called to other Enduro products, such as Enduro 18–8, a chromium-nickel steel. The present experiment refers to Enduro Type "A" only.

Liquids used. The liquids used consisted of suitable solutions of such dairy washing powders as special alkali, sodium carbonate, Wyandotte, tri-sodium phosphate, Tin Cleaner, Diversol, and sodium hydroxide; of chemical sterilizers such as sodium hypochlorite, Diversol, and chloramine-T; of neutralizing lime; and of refrigerating brines, both calcium chloride and sodium chloride, variously treated for protection against corrosion.

Method of procedure. One-half pint Mason jars with glass covers were used in all the experiments, each jar containing 130 cc. of the liquid under study. This amount of liquid was just sufficient to cause the 4 inch strip to be one-half immersed in the liquid. The surface exposed to the liquid was 5.5 square inches. In the case of the washing powders the time and temperature of exposure was 5 hours at 150°F., and for the chemical sterilizers it was 5 days at 70°F. In the case of brine the strips were immersed in cold brine and held in a cooler at approximately 32°F. for ten days. A special experiment was also conducted in which the strips were first steamed for 2 hours, then held in cold water for 2 hours, then in cold $CaCl_2$ brine for 2 hours, and then removed from the brine, and these brine-wet strips were then held in empty, open bottles overnight. This treatment was repeated daily for seven consecutive days. Prior to use the metal strips were cleaned by washing them in a very dilute solution of trisodium phosphate. They were then thoroughly rinsed in water, wiped dry, dried in a vacuum oven and carefully weighed. At the end of the period of exposure the strips were again washed, rinsed, dried, and weighed as before.

METALS IN WASHING POWDER SOLUTIONS

The washing powders were used in the form of the following solutions:

0.5 per cent solution of sodium hydroxide
0.5 per cent solution of special alkali consisting of approximately 35 per cent NaOH and 62 per cent Na_2CO_3
0.5 per cent solution of sodium carbonate
0.5 per cent solution of Wyandotte Cleaner and Cleanser consisting of approximately 49 per cent Na_2CO_3 and 36 per cent $NaHCO_3$
0.5 per cent solution of tri-sodium phosphate
0.16 per cent solution of tri-sodium phosphate
0.5 per cent solution of tin cleaner, consisting of approximately 95 per cent Na_3PO_4 and 5 per cent Na_2CrO_4

The time and temperature of exposure of all solutions was 5 hours at 150°F. except in the case of sodium hydroxide and tin cleaner with which additional tests were also made, with an exposure for 5 days at 70°F. An additional series of tests with specially treated washing solutions was made in the case of the aluminum products as described in later paragraphs.

At the conclusion of these tests, the change in weight of the metal strips, the visible corrosion on the strips and the appearance of the liquid were observed. The results are given in tables 1, 2, and 3. Figure 1 shows the appearance of the metal strips after immersion in some of the solutions under study.

Discussion of results shown in tables 1, 2, and 3. In table 1 are shown the total weight losses of all the metals arranged according to washing solutions, and the losses suffered by the aluminum products and by the tinned metals separately. Tables 2 and 3 give the individual weight losses and visible corrosion, respectively, for each metal.

TABLE 1

Total weight losses of metal strips in washing sodas

WASHING POWDER SOLUTIONS	TOTAL LOSS IN WEIGHT OF ALL METALS	LOSS IN WEIGHT OF ALUMINUM PRODUCTS	LOSS IN WEIGHT OF TIN-COATED METALS	TOTAL LOSS IN WEIGHT OF ALL METALS EXCEPT ALUMINUM PRODUCTS AND TIN-COATED METALS
	mgm.	*mgm.*	*mgm.*	*mgm.*
Sodium hydroxide 0.5 per cent 5 hours 150°F.	904 3	720 7	129 1	54.5
Sodium hydroxide 0.5 per cent 5 days 70°F.	4,297 6	3,507 7	665 9	124 0
Special alkali 0.5 per cent 5 hours 150°F.	481.9	371 0	81.1	29.8
Sodium carbonate 0.5 per cent 5 hours 150°F.	193 9	98 0	64 9	31 0
Wyandotte 0.5 per cent 5 hours 150°F.	153 6	106 6	36 4	10 6
Tri-sodium phosphate 0.5 per cent 5 hours 150°F.	227 2	155 6	65 9	5 7
Tri-sodium phosphate 0.16 per cent 5 hours 150°F.	86 3	39 5	32 0	14.8
Tri-sodium phosphate 0.5 per cent with 0.025 per cent chromate 5 hours 150°F.	80.0	61 4	3 7	14 9

The corrosion and weight losses totaled greatest in washing solutions containing caustic soda, such as the solutions of sodium hydroxide and of special alkali, as shown in table 1. The next greatest total losses occurred in the case of 0.5 per cent solutions

TABLE 2

Loss in weight of metal strips after immersion in washing powder solutions; partial immersion

METALS	SODIUM HYDROXIDE		ALKALI SPECIAL, 5 HOURS, 150°F., 0.5 PER CENT SOLUTION	SODIUM CARBONATE, 5 HOURS, 150°F., 0.5 PER CENT SOLUTION	WYANDOTTE, 5 HOURS, 150°F., 0.5 PER CENT SOLUTION	TRI-SODIUM PHOSPHATE		TIN-CLEANER	
	5 hours, 150°F., 0.5 per cent solution	5 days, 70°F., 0.5 per cent solution				5 hours, 150°F., 0.5 per cent solution	5 hours, 150°F., 0.16 per cent solution	5 hours, 150°F., 0.5 per cent solution	5 days, 70°F., 0.5 per cent solution
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Single metals:									
Aluminum	317.4	453.0	141.8	35.3	36 7	52.6	13.4	19.0	0.5
Copper	0.0	87.0	0.7	0.1	0 0	0 5+	0.2+	0 4	0.3
Iron	10.5	3.8	2.5	11.2	2 6+	0.9	1 3	4.2	1.9
Nickel	0.6	1.1	0 5	0.6	0 0	0.0	0 5+	1 3	0.0
Tin	19.7	0.4	16.9	10.9	0 8	0.2	9 1	1.0	0.4
Zinc	6.7	8.4	3.6	2 9	4.2	1.8	2 2	1.4	1.6
Plated metals:									
Copper, tinned E. B.*	22.1	141.7	17.1	16.3	9.7	7.8	0.3+	0.2	0.2+
Copper, tinned E. T.†	23 4	187.7	9 5	8.2	8.4	9.0	9.0	0.3	0.0
Copper Retin	37.8	9.1	29.3	18 5	10 5	29.4	12.0	0.7	0.2
Iron, galvanized	15.3	20.7	3.2	2 8	1.8	0.4+	0.1	2.6	0.5+
Iron, tinned E.B.*	20.0	259.0	12 2	10.0	1.0	9 3	0 8	0.2	0.4+
Iron, tinned E.T.†	25 8	68.4	13 0	11 9	6.8	10.4	9.9	2.3	0.9
Al. alloy, Al. plated	216.3	1,432.7	136.0	35.1	34.8	51.4	13.6	25.1	5.0
Alloys:									
Ascoloy	0.0	0 4	0.4	0.2	0.0	0.3+	0.1	1.1	0.0
Allegheny Metal	0.0	0.1	0.4	0.1	0.0	0.0	0.0	0.5	0.0
Enduro	0 0	0.0	0.8	0.1	0.0	0 5	0 6+	1.0	0 0
Monel Metal	0.3	0.8	0.0	0.6	0 3+	0.7+	0.2+	0.3	0.0
Nickel silver	1.4	1.3	0.8	1.5	0 9	0.4	0.5+	1.1	0.7
Mn. Al. alloy	187.0	1,622.0	93 2	27.6	35.1	51.6	12.5	17.3	13.4

* Edges bare (not tinned).

† Edges tinned.

+ indicates gain in weight.

Appearance of metal strips after immersion in washing powder solutions; partial immersion

METALS	SODIUM HYDROXIDE		ALKALI SPECIAL, 5 HOURS, 150°F., 0.5 PER CENT SOLUTION	SODIUM CARBONATE, 5 HOURS, 150°F., 0.5 PER CENT SOLUTION	WYANDOTTE, 5 HOURS, 150°F., 0.5 PER CENT SOLUTION	TRI-SODIUM PHOSPHATE		TIN CLEANER
	5 hours, 150°F., 0.5 per cent solution	5 days, 70°F., 0.5 per cent solution				5 hours, 150°F., 0.5 per cent solution	5 hours, 150°F., 0.16 per cent solution	5 hours, 150°F., 0.5 per cent solution
Single metals.								
Aluminum	t ccc	O.K. cccc	t cccc	t ccc	t ccc	t tttt	c ccttt	t ct
Copper	O.K. ttt	t cctt	O.K. tttt	ttt ttt	t tttt	O.K. ttt	tt c	O.K.
Iron	ccc c	O.K. ccc	c O.K.	cc O.K.	c O.K.	c O.K.	cc c	c O.K.
Nickel	t O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	t O.K.	O.K.
Tin	t ccc	t c	t ccct	t ccct	t O.K.	t O.K.	O.K. ccc	O.K.
Zinc	t ccc	cc ccc	c c	tt cc	tt cc	t O.K.	tt c	O.K.
Plated metals:								
Copper, tinned E. B.	O.K. ccc	O.K. ccc	t ccc	t cc	t cc	t cc	tt O.K.	O.K.
Copper, tinned E. T.	O.K. ccc	t cccc	O.K. ccc	O.K. ccc	tt cc	t ccc	O.K. ccc	t O.K.
Copper Retin	O.K. ccc	t ccc	t ccc	t ccc	t ccc	t ccc	O.K. ccc	O.K.
Iron, galvanized	O.K. ccc	c ccc	O.K. c	O.K. c	t cc	O.K. c	O.K.	O.K.
Iron, tinned E. B.	c ccc	O.K. cccc	t ccc	t ccc	t O.K.	t cc	O.K.	c O.K.
Iron, tinned E. T.	O.K. ccc	O.K. ccc	t ccc	c ccc	c cc	O.K. cc	O.K. cc	c O.K.
Al alloy, Al plated	O.K. cccc	O.K. cccc	c cccc	O.K. cc	O.K. cc	O.K. tttt	t ccttt	O.K. ttt
Alloys								
Ascoloy	t O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.
Allegheny Metal	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.
Enduro	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.
Monel Metal	t c	O.K.	O.K.	O.K.	O.K.	O.K.	t O.K.	O.K.
Nickel silver	t c	t O.K.	O.K.	t O.K.	O.K. t	tt O.K.	tt O.K.	tt O.K.
Mn. Al. alloy	O.K. cccc	O.K. cccc	c cccc	t ccct	O.K. cct	O.K. tttt	O.K. ccttt	t tt

O.K. = no change.

c = slightly corroded, cc = corroded, ccc = heavily corroded, cccc = very heavily corroded.

t = slightly tarnished, tt = tarnished, ttt = heavily tarnished.

The condition of the strip above and below the air-liquid line is indicated by the position of the key letter above or below the dash. Thus, tarnished above and corroded below the air-liquid line is indicated as follows: $\frac{tt}{cc}$, heavily tarnished above and heavily corroded below $\frac{ttt}{ccc}$, etc.

of tri-sodium phosphate and of sodium carbonate. Wyandotte ranked next, while the 0.16 per cent solutions of tri-sodium phosphate and the 0.5 per cent solutions of tri-sodium phosphate treated with sodium chromate gave the greatest protection to the metals.

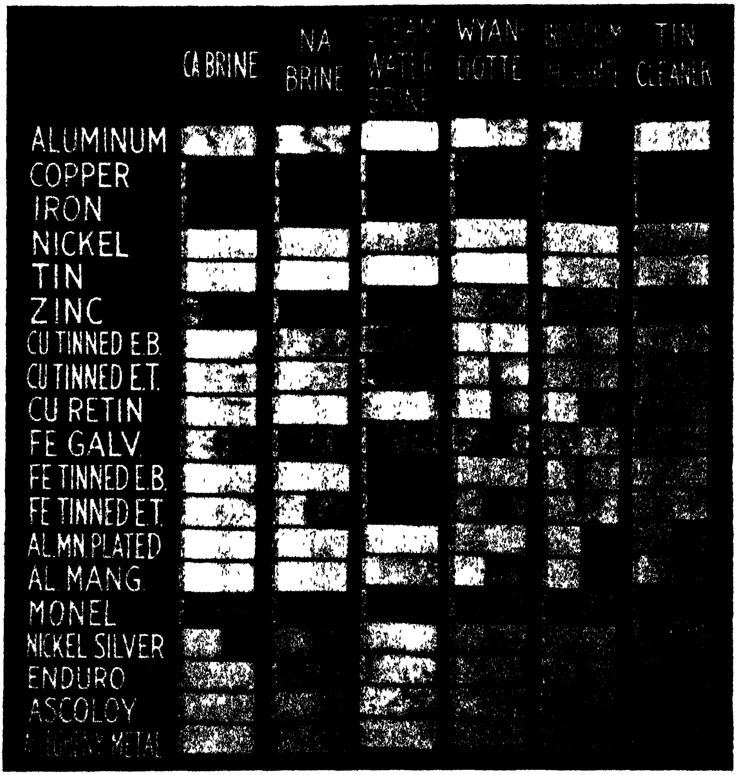


FIG. 1. METAL STRIPS IN SOME OF THE WASHING SOLUTIONS AND BRINES

In considering these total weight losses it should be noted that the large differences in these figures between the various washing sodas are due chiefly to the high weight losses of the aluminum products and the tin-coated metals. Eliminating the losses suffered by these metals reveals the fact that the remaining metals, as a whole proved comparatively resistant to attack by washing sodas.

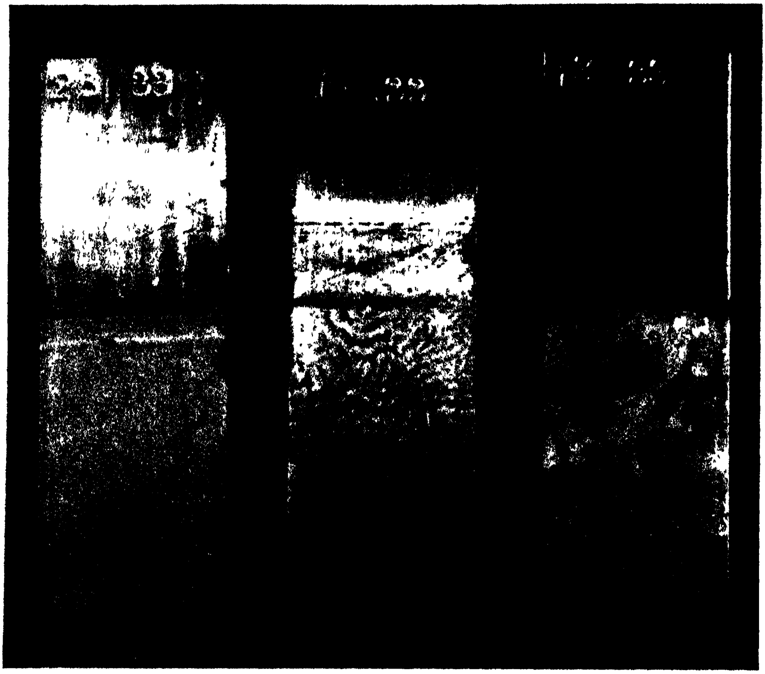
Thus, as indicated in tables 2 and 3, the chromium-nickel steel, Allegheny Metal, and the ordinary chromium steels, Ascoloy and Enduro, were practically free from attack. There was no visible corrosion and no discoloration on these strips, and the weight losses, where there were any, were negligible. The liquids, themselves, also were free from precipitation. Similarly nickel and Monel Metal showed very high resistance. In the case of nickel silver the weight losses were also very small, but these strips suffered marked tarnishing in some of the liquids. Copper in most instances lost but little in weight; however, it tarnished very profusely, suggesting that corrosion may have set in, but that the resulting weight losses were offset, in part at least, by oxide formation on the surface. Iron and galvanized iron were intensely attacked in the caustic sodas but to a somewhat lesser extent in the other washing compounds. The resistance of tin lacked in uniformity but was far greater in most cases than that of tinned copper and tinned iron. Zinc showed the least resistance of the metals outside of the aluminum products and the tin-coated products. The greater corrosion of the tin-coated metals than tin itself suggests the probability of electrochemical effect, the tin being electropositive to the copper and electronegative to the iron. This suggestion is further strengthened by the galvanized appearance of the tinned metal strips, while the all-tin strips lacked this characteristic appearance. The effect here was to cause the tinned copper and tinned iron strips to resemble the surface of galvanized iron, while this change in appearance was entirely absent on the all-tin strips, although the latter showed marked but uniform corrosion, as shown in figure 2.

North (2) who studied the attack on metals in much stronger solutions of alkaline washing powders, i.e., 3 per cent solutions of sodium hydroxide, of Oakite, and of Wyandotte, reports similar results as far as the resistance of individual metals and alloys is concerned. Thus, he states that "In these tests made with alkali washing powders, all of the metals[1] showed remarkable resistance, with the exception of tin-plated steel. The effect of

---

[1] These tests did not include tinned copper nor aluminum.

Oakite, Wyandotte, and even sodium hydroxide was very slight on the stainless steels (meaning both ordinary chromium steels and chromium-nickel steel) and on nickel and Monel Metal. On the other hand, the tin plate showed much greater corrosion to all of these alkali preparations."



Pure tin Tinned copper Tinned iron

FIG. 2. SHOWING GALVANIZED EFFECT OF SODIUM CARBONATE ON TIN PLATED METALS

Our experiments further demonstrated that the corrosion caused by tri-sodium phosphate may be very greatly reduced by the addition to the solution of a very small amount of sodium chromate (0.025 per cent Na_2CrO_4 in the washing solution). This was the case even in as strong a washing soluti~~on as a 0~~.5 per cent solution of tri-sodium phosphate. This corrosion-protective effect was very marked in the case of the aluminum products and was especially great in the case of the tinned metals.

The weight losses of the tin-coated metals in chromate-treated tri-sodium phosphate solutions totaled approximately one-twentieth only of the losses in the same strength solution without chromate treatment.[2]

Considering the corrosion results given in these experiments cognizance should be taken of the fact that the several washing powders were used in the form of 0.5 per cent solutions. In addition to this 0.16 per cent solutions of tri-sodium phosphate were also employed. It is obvious that these washing powders differ from one another in their alkalinity and causticity and that, therefore, they should not be and in fact are not used in solutions of the same percentage strength by weight. Thus, in practical plant operation it is customary to use from 0.25 to 0.5 per cent solutions of Wyandotte Cleaner and Cleanser, approximately one-half of this concentration of sodium carbonate, and about one-third in the case of tri-sodium phosphate. These proportions have been found most suitable for general cleaning operations and approximately equal in cleaning power.

In their corrosive action on tin-coated metals also they have approximately the same relation to each other, as shown in table 1. Thus, 0.5 per cent solutions of sodium carbonate caused nearly twice the weight losses of 0.5 per cent solutions of Wyandotte, and 0.16 per cent solutions of tri-sodium phosphate yielded about the same weight losses as 0.5 per cent Wyandotte solutions, while the metal weight losses in the 0.5 per cent solutions of special alkali totaled considerably larger.

The corrosiveness of the stronger tri-sodium phosphate solutions, however, was reduced almost to a negligible effect by addition to these solutions of 0.025 per cent of sodium chromate, as previously pointed out. In a like manner the addition to special alkali of 5 per cent sodium chromate reduced the corrosive action on tin-plated iron approximately 50 per cent. The special alkali solution here, as in the case of chromate-treated tri-sodium phosphate, contained 0.025 per cent chromate of soda. Thus, in a separate set of tests with tinned iron the metal strips in the untreated 0.5 per cent solution of special alkali averaged a loss of 9.2 mgm., while in the chromate-treated portion of the same solution the weight losses averaged 4.2 mgm.

---

[2] This preparation, containing principally a combination of tri-sodium phosphate and sodium chromate is patented by the Diversey Mfg. Co., who also claim to be its originators. It is manufactured and sold by them under the trade name Protex (metallum). In the present article this preparation is recorded under the name "Tin Cleaner" which was its name at the inception of this experiment.

These results emphasize the importance of careful consideration in the selection of washing powders, especially for cleaning tin-coated dairy equipment, such as milk and cream shipping cans and tinned copper surfaces in vats, etc. Thus, the use of caustic alkalies rapidly destroys the tin coating while the use of chromate-treated tri-sodium phosphate solutions assists materially in solving the problem of preserving the tin coating on such equipment.

Referring to table 2, it will be noted that the weight losses for aluminum and aluminum products were greater than those for any other metal. This holds true in the case of every washing powder studied, and a comparison of the total corrosion weight losses of all the metals in all the washing powders, see table 1, with the losses of the three aluminum products, shows that the weight losses for the aluminum products constituted over two thirds of the total weight losses of all the metals combined. Because of this intense corrosion of aluminum in alkalies, a special series of tests was made with alkaline washing powders variously treated for the purpose of determining to what extent such treatment may be depended upon to protect aluminum products effectively against corrosion.

The several washing powders were treated with sodium silicate and sodium chromate, respectively. These results are given in table 4.

Discussion of results shown in table 4. Similarly as in the case of the results shown in tables 1, 2, and 3, so in this additional experiment, the aluminum products suffered very severe attack in the untreated alkaline washing powders. Here, too, the attack on the manganese aluminum alloy was practically equal to that on the pure aluminum. In the case of sodium hydroxide, the addition of sodium silicate failed to exert any corrosion inhibiting

TABLE 4

Effect of washing sodas on aluminum and manganese aluminum alloy; 150°F. for 5 hours

WASHING POWDERS	ALUMINUM		MANGANESE ALUMINUM ALLOY	
	Loss in weight	Appearance	Loss in weight	Appearance
	mgm.		*mgm.*	
Sodium hydroxide 0.5 per cent	163.0	Very heavy corrosion and pitting, metal heavily covered with oxide	95.4	Heavy corrosion, metal heavily covered with oxide
Sodium hydroxide 0.5 per cent with silicate 0.05 per cent	433.0	Very heavy corrosion, absence of oxide, metal bright	404.0	Very heavy corrosion, absence of oxide, metal dark colored
Sodium carbonate 0.5 per cent	24.9	Corroded, metal covered with white deposit	41.1	Corroded, metal covered with brown deposit
Sodium carbonate 0.5 per cent with silicate 0.05 per cent	0.9+	Tarnished	0.6+	Tarnished
Tri-sodium phosphate 0.16 per cent	17.7	Slightly corroded and heavily tarnished, black color	16.1	Slightly corroded and heavily tarnished, black color
Tri-sodium phosphate 0.16 per cent with silicate 0.05 per cent	19.6	Slightly corroded and heavily tarnished, black color	18.9	Slightly corroded and heavily tarnished, black color
Tri-sodium phosphate 0.5 per cent	52.6	Corroded and heavily tarnished, black color	51.6	Corroded and heavily tarnished, black color
Tri-sodium phosphate 0.5 per cent with chromate 0.025 per cent	19.0	Slightly corroded and heavily tarnished, gray color	17.3	Slightly corroded and heavily tarnished, gray color

effect, in fact, the attack on the metal was greatly augmented by such treatment.

The treatment of sodium carbonate with sodium silicate, however, eliminated corrosion entirely, the weight changes being negligible and the metal strips showing only a slight tarnish. The addition to tri-sodium phosphate of silicate of soda did not diminish the corrosiveness of this washing solution, nor did it eliminate the blackening of the aluminum product which was characteristic of all tri-sodium phosphate solutions on aluminum, and which is very objectionable. The treatment of a 0.5 per cent solution of tri-sodium phosphate with sodium chromate, while it markedly lessened the corrosion and weight losses which occurred in the same strength solution without chromate treatment, failed to eliminate corrosion entirely. The weight losses here were still considerable and the metal strips showed a heavy, grayish tarnish, instead of the jet black tarnish.

These findings are supported also by Seligman (3) who states that "soda is detrimental if used without precautions." He even goes so far as to advise that, "soda can be used with complete impunity if a small quantity of silicate of soda is incorporated with it." Churchill (4), however, cautions that "the real efficacy of soda silicate as an inhibitor of corrosion is shown where used with normal sodium carbonate. The silicate does not work well with bicarbonate, due to the precipitation of a gummy mass of silica, incident to the neutralization of the bicarbonate to normal carbonate. The inhibitor is also effective with borax, soap, etc., and in many cases where the detergent is made of a strong base (sodium) with a weak acid (carbonic, boric, stearic, etc.)."

It is evident from our results that the only washing solution that furnishes effective protection against damaging attack of the aluminum is silicate-treated sodium carbonate. Sodium carbonate solutions containing 0.05 per cent sodium silicate, as used in this experiment, are, therefore, recommended for the cleaning of aluminum equipment. For the cleaning of tin-coated equipment, on the other hand, the use of tri-sodium phosphate containing a small amount of sodium chromate (0.025 per cent Na_2CrO_4) appears to afford the best protection.

Partial immersion versus total submersion. Another significant point revealed in these experiments that has a direct bearing on the control of corrosion in dairy equipment, is that where the metal strips were totally submerged the corrosion was far less than in the case of partial immersion. These results are given in table 5.

TABLE 5

Comparison of total immersion and half immersion; weight losses in washing solutions

METALS	TRI-SODIUM PHOSPHATE, 0.5 PER CENT		SODIUM CARBONATE, 0.5 PER CENT	
	Total immersion	Half immersion	Total immersion	Half immersion
	mgm.	*mgm.*	*mgm.*	*mgm.*
Single metals:				
Aluminum	44.1	52.6	18.3	35.3
Copper	0.2	0.5+	0.1	0.1
Iron	0.0	0.9	0.0	11.2
Nickel	0.0	0.0	0.3	0.6
Tin	0.3+	0.2	3.4	10.9
Zinc	0.2	1.8	1.1	2.9
Plated metals:				
Copper, tinned E. B.	0.4	7.8	1.6	16.3
Copper, tinned E. T.	6.8	9.0	1.2	8.2
Copper Retin	4.9	29.4	5.9	18.5
Iron, galvanized	0.4+	0.4+	0.7	2.8
Iron, tinned E. B.	7.7	9.3	6.4	10.0
Iron, tinned E. T.	6.5	10.4	1.7	11.9
Al. alloy, Al. plated	41.9	51.4	25.3	35.1
Alloys:				
Ascoloy	0.4+	0.3+	0.2+	0.2
Allegheny Metal	0.8+	0.0	0.2+	0.1
Enduro	0.7+	0.5	0.3	0.1
Monel Metal	0.0	0.7+	0.0	0.6
Nickel silver	0.2+	0.4	0.0	1.5
Mn. Al. alloy	42.4	51.6	5.8	27.6
Total weight losses	157.9	227.2	72.5	193.9

Table 5 shows that wherever there was considerable loss of weight in the case of the completely submerged strips, the weight losses were greatly increased in the case of the half-immersed strips. The appearance of the strips suggested that the greater weight losses for the half-immersed strips were due in part to intensified corrosion at the air-liquid line and in part to added corrosion of the portion projecting above the liquid. These findings are significant in their relation to practical plant operation. They suggest that the prolonged holding of washing solutions in vats, etc., during cleaning operations and the incomplete removal of washing solutions from metal surfaces may greatly magnify the corrosive action on metal surfaces and may especially also tend to shorten the life of the tin coating in tinned metal equipment. These findings are corroborated by many experiences and observations in the operation and cleaning of milk plant equipment.

METALS IN CHEMICAL STERILIZERS

Most of the chemical sterilizers used in connection with the cleaning of dairy equipment depend for their sterilizing effect on the presence of available chlorine. Since free chlorine is an active corrosive agent, these chemical sterilizers may constitute an important factor in the corrosion of equipment. These facts emphasize the importance of their careful consideration in efforts to preserve the life of metal surfaces.

The chemical sterilizers used consisted of solutions of sodium hypochlorite (1 ounce per gallon), Diversol (0.64 ounce per gallon and 1.1 ounces per gallon respectively), and chloramine-T (0.1 ounce per gallon. In addition the effect on the metals of a neutralizer mix of hydrated calcium magnesium lime, containing two pounds of dry lime per gallon of liquid mix, was studied. The temperature and time of exposure in all liquids was 70°F. for 5 days. The metal strips were immersed in these liquids to the extent of half of their lengths and all observations were made in accordance with the "Method of Procedure" previously given. The results are shown in tables 6 and 7.

Discussion of results shown in tables 6 and 7. In table 6 are recorded the weight losses of the individual metals in the several solutions and table 7 gives the appearance of the metal strips after exposure. These results show that the hypochlorite solutions were by far the most corrosive of the chemical sterilizers

TABLE 6

Loss in weight of metal strips in solutions of chemical sterilizers; partial immersion

METALS	SODIUM HYPOCHLORITE, 5 DAYS, 70°F., 1 OUNCE, PER GALLON*	DIVERSOL, 5 DAYS, 70°F.		CHLORAMINE-T, 5 DAYS, 70°F., 0.1 OUNCE PER GALLON§	NEUTRALIZING LIME MIX, 5 DAYS, 70°F., 2 POUNDS PER GALLON‖
		1.1 ounce per gallon†	0.64 ounce per gallon‡		
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm*
Single metals:					
Aluminum	0.8	53.5	33.0	0.2	149.0+
Copper	1.1+	0.1	0.5	0.6	18.7
Iron	54.9	0.1	1.6	53.7	27.3
Nickel	7.0	0.5+	0.9	0.4+	0.2+
Tin	29.8	0.3	0.5+	2.5	0.4
Zinc	38.5	8.6	10.5	30.1	13.1
Plated metals:					
Copper, tinned E. B.	34.1	0.6	0.6	2.4	1.8
Copper tinned E. T.	13.0	0.2+	0.9	1.2	7.8
Copper Retin	9.3	0.8	0.3	1.7	4.7
Iron, galvanized	36.7	0.2+	13.0	0.0	9.3
Iron, tinned E. B.	20.2	0.2+	0.7	14.3	4.6
Iron, tinned E. T.	8.0	1.3	1.4	6.1	4.2
Al. alloy, Al. plated	2.2	65.7	33.5	0.1	208.8+
Alloys:					
Ascoloy	0.2+	0.6	1.2	0.8+	0.4
Allegheny Metal	0.0	0.3	0.9	1.6	0.0
Enduro	0.0	0.3	0.7	0.5	0.4+
Monel Metal	8.2	0.5	0.3	0.6	1.1+
Nickel silver	6.0	0.4	1.0	0.7	1.6
Mn. Al. alloy	5.6	52.6	30.4	0.4	185.0+

* This solution contains 236 parts available chlorine per million.

† This solution contains 236 parts available chlorine per million.

‡ This solution contains 137 parts available chlorine per million.

§ This solution contains 222 parts available chlorine per million.

‖ This solution is made from calcium-magnesium hydrated lime containing 66.5 per cent $Ca(OH)_2$ and 32.5 per cent MgO.

studied. The only metals not affected by hypochlorite solutions were Allegheny Metal and Enduro. Ascoloy also suffered only very slight attack. Aside from these chromium steels all the

TABLE 7

Appearance of metal strips in solutions of chemical sterilizers; partial immersion

METALS	SODIUM HYPOCHLORITE, 5 DAYS, 70°F., 1 OUNCE PER GALLON	DIVERSOL, 5 DAYS, 70°F.		CHLORAMINE-T, 5 DAYS, 70°F., 0.1 OUNCE PER GALLON	NEUTRALIZING LIME MIX, 5 DAYS, 70°F., 2 POUNDS PER GALLON
		1.1 ounce per gallon	0.64 ounce per gallon		
Single metals:					
Aluminum	O.K. cc	O.K. cct	c cct	t ct	t cccc
Copper	tt cttt	t O.K.	O.K. c	O.K. c	t cc
Iron	cc ccc	cc cc	O.K. c	O.K. ccc	cc O.K.
Nickel	t c	O.K. tt	O.K.	O.K.	O.K.
Tin	cc c	t c	O.K.	O.K. c	O.K.
Zinc	ccc ccc	cc c	t O.K.	O.K. ccc	O.K. cccc
Plated metals:					
Copper, tinned E. B.	ccc ccc	t t	tt t	t t	O.K. c
Copper, tinned E. T.	ccc c	t t	tt O.K.	t c	O.K. cc
Copper Retin	cc c	O.K.	t O.K.	t c	O.K. cc
Iron, galvanized	c ccc	cc O.K.	O.K. cc	O.K. ccc	c cc
Iron, tinned E. B.	cc ccc	O.K.	c O.K.	c c	O.K.
Iron, tinned E. T.	cc cc	c c	c O.K.	c cc	c t
Al. alloy, Al. plated	cc cc	O.K. cct	c ttt	O.K. t	t cccc
Alloys:					
Ascoloy	c O.K.	t O.K.	O.K.	O.K.	O.K.
Allegheny Metal	O.K.	O.K.	O.K.	O.K.	O.K.
Enduro	O.K.	O.K.	O.K.	O.K.	O.K.
Monel Metal	O.K. cc	O.K. t	O.K.	O.K.	O.K.
Nickel silver	ttt ccc	O.K. t	O.K.	ttt O.K.	O.K.
Mn. Al. alloy	O.K. ccc	O.K. ccct	cc ttt	O.K. ct	cc cccc

metals studied suffered more or less intense corrosion and weight losses in this chlorine solution. Iron, zinc, galvanized iron, tin, and the tin-plated products in the order named here incurred the greatest weight losses and corrosion; nickel, nickel silver, and Monel Metal also were severely attacked, while copper and the aluminum products suffered the least.

The Diversol solutions, the chlorine content of which also is present in the form of sodium hypochlorite, but which in addition contains approximately 94 per cent of tri-sodium phosphate, proved far less corrosive than the straight hypochlorite. With the exception of the losses that occurred in the case of the aluminum products the total losses in the Diversol were comparatively small. Being an alkaline product, Diversol did, however, severely attack the aluminum products. Other metals that suffered appreciable weight losses and corrosion in the Diversol solutions were zinc and galvanized iron.

In the chloramine-T solutions iron, zinc, and tinned iron corroded by far the most. Tin and the tinned copper products showed much smaller weight losses and the visible corrosion was far less pronounced. The weight losses of the aluminum products, nickel, copper, Monel Metal, nickel silver, and the chromium steels were negligible but there was some visible corrosion of the aluminums and of the copper strips, and some tarnish on the nickel silver strips, while the nickel, Monel Metal, and the three chromium steels, Allegheny Metal, Ascoloy, and Enduro, were entirely free from visible attack. In the case of galvanized iron there were no weight losses, but the metal strip showed heavy corrosion, indicating that while marked corrosion occurred, the accumulation of oxidation products on the surface compensated for the weight loss caused by the corrosion.

A comparison of these results with chemical sterilizers suggests that the hypochlorite exerted by far the greatest corrosive action on the metals, while the weight losses in the case of chloramine-T totaled least. In this comparison it should be pointed out, however, that the weight losses in the case of Diversol were largely confined to the heavy corrosion of the aluminum products in the presence of the alkaline tri-sodium phosphate. Eliminating the aluminum products from these totals the Diversol stands out as by far the least corrosive chemical sterilizer. This is true especially also in the case of the tinned products.

Allegheny Metal and Enduro showed no visible attack in any of these solutions. These were closely followed by Ascoloy, nickel, Monel Metal, and nickel silver, in the order named. These results are also supported by North (2) who found Allegheny Metal and Enduro highly resistant to hypochlorite solutions. His results, however, show nickel and Monel Metal to be more corrosive than tin-plated steel in very strong calcium hypochlorite solutions. Quam (5) also found Allegheny Metal and high chromium steel to withstand attack of sodium hypochlorite.

In the neutralizing lime nickel, tin, Monel Metal, nickel silver, and the three chromium steels showed no corrosion and the weight losses were either negligible or entirely absent. The tinned copper and tinned iron products suffered considerable weight losses and in most cases there was visible corrosion. Iron, copper, zinc, and galvanized iron were severely attacked. The aluminum products were very intensely corroded and in addition showed a heavy accumulation of aluminum hydroxide on the surface, which may account for the extraordinary increase in weight in the case of these products.

METALS IN BRINES

As previously stated the suitability of metals in dairy equipment is not confined alone to their behavior in milk products and washing solutions but is reflected also in their resistance to corrosion in the presence of refrigeration brine.

In milk plant operation the sodium chloride brine and the calcium chloride brine are the principle brines used. When first made the sodium brine is usually neutral in its reaction while calcium brine is slightly alkaline. When in circulation for refrigeration, however, these brines suffer continuous exposure to air. This is accompanied by absorption of carbon dioxide from the air, even in the so-called closed systems; this causes the brine to gradually become acid in its reaction.

Thus, in brine systems, in the installation and operation of which no provision was made to guard against contact of the brine with air, and where no attempt was made to control the reaction of the brine by systematic neutralization of its acidity, the authors found such brines invariably acid after being in use even for a comparatively short period of time. In another large brine system in daily use, which was also under our observation, the alkalinity of the brine, after having been brought up to 0.05 per cent by addition of NaOH gradually decreased to 0.016 per cent in the course of a year's time.

This increased acidity intensifies the corrosive action of the brine. Hence the degree of acidity that is permitted to accumulate in the brine obviously plays an important rôle in the resistance of the various metals exposed to such brines. The continued operation of steel-jacketed milk and cream tanks used for brine cooling furnishes a striking example of this fact. The jacket side of these tanks invariably yields to intense corrosion, even to the extent of causing leaks, and large quantities of rust accumulate on the steel surfaces and on the bottom of the jackets, retarding heat and cold transmission. This difficulty has long been recognized by refrigerating engineers. Efforts to minimize exposure of the brine to air in the circulating system are desirable and helpful, but experience has shown that this does not completely prevent absorption of carbon dioxide by the brine and that the acidity does continue to increase.

In the present experiment our study of the resistance of the 19 different metals to brine included both neutral brines and brines specially treated for corrosion diminution, such as by the addition of alkalies, of sodium chromate, and of sodium silicate, respectively.

Both the sodium and the calcium brines were standardized to a specific gravity of 1.17, made neutral to phenolphthalein, and divided into four parts each. One part of each brine was used untreated, to the second part was added enough sodium hydroxide to attain an alkalinity of 0.05 per cent NaOH, to the third portion was added 0.17 per cent sodium chromate, and to the fourth 0.13 per cent of a 40° Be. solution of sodium silicate.

In an additional experiment an effort was made to simulate as

TABLE 8

Loss in weight of metal strips in brines; partial immersion for 10 days at 33°F.

METALS	CALCIUM BRINE, SPECIFIC GRAVITY 1.17				SODIUM BRINE, SPECIFIC GRAVITY, 1.17				SUCCESSIVE STEAM-WATER-BRINE-AIR TREATMENT*
	Neutral	Alkaline, 0.05 per cent $Ca(OH)_2$	Chromate, 0.17 per cent Na_2CrO_4	Silicate, 0.13 per cent 40°Be.Sil.	Neutral	Alkaline, 0.05 per cent NaOH	Chromate, 0.17 per cent Na_2CrO_4	Silicate, 0.13 per cent 40°Be.Sil.	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Single metals:									
Aluminum	0.4	15.6+	0.4+	0.8	3 3	40.6	0.0	1.0+	32.0+
Copper	16.0	14.9	9.8	12.2	13.9	0.7	7.3	12.4	1.7+
Iron	14.4	26.3	11.0	11.5	29.5	20.9	21.9	11.0	321.8
Nickel	0.0	0.2	0.7	0 5	0.9	0.0	0.4	0.0	1.4+
Tin	3.6	4.7	2.0	2.8	2.9	6.7	1.7	2.5	0.9
Zinc	11.9	9.0	7.6	8.7	17.7	43 0	2.5	35.1	0.8+
Plated metals:									
Copper, tinned E. B.	4.2	10.5	1.7	3.4	5.9	6.2	0.9	4.2	3.0
Copper, tinned E. T.	7.8	7.2	1.3	6.3	8.1	33.1+	2.0	0.2	2.1
Copper Retin	6.9	0.1+	0.3	2 5+	1.3	73.3	1.9	0.2	1.1
Iron, galvanized	7.8	14 7	6.6	13.1	4.6	54.2	24 8+	70.9	194.3+
Iron, tinned E. B.	9.7	5.3	1.9	6.7	7.5	4.1	3.3	2.4	12.3+
Iron, tinned E. T.	3.4	9.3	1.8	3.6	4.9	41.5	2 2	2.2	14.1
Al. alloy, Al. plated	0.2	21.3+	0.4+	0.7	2.9	27.1	0.0	0.8+	18.4+
Alloys:									
Ascoloy	6.0	0.5	0.8	0 7	2.7	0.4	0.1+	0.0	0.9+
Allegheny Metal	0.0	0.0	0 5	0.0	1.0	0.8+	0.0	0.0	0.6+
Enduro	0.0	0.0	0 0	0.2	1.4	0.7	0.0	0.5	7.5+
Monel Metal	0.5	0.2+	1.4	10.2	2 9	0.6+	0.9	0.7+	7.7+
Nickel silver	0.7	0.5	0.8	0.6	5 8	0.2	1.0	0.3+	2.4+
Mn. Al. alloy	0.6	21.9+	0.2+	1.1	2.8	22.6	0.0	0.1+	28.6+

* Complete submersion; two-hour exposure each in steam, in water, in brine, and 18 hours in air; treatment repeated daily for seven consecutive days.

TABLE 9

Appearance of metal strips after immersion in brine; partial immersion for 10 days at 33°F.

METALS	CALCIUM BRINE, SPECIFIC GRAVITY, 1.17				SODIUM BRINE, SPECIFIC GRAVITY 1.17				SUCCESSIVE STEAM-WATER-BRINE-AIR TREATMENT
	Neutral	Alkaline, 0.05 per cent $Ca(OH)_2$	Chromate, 0.17 per cent Na_2CrO_4	Silicate, 0.13 per cent 40°Be.Sil.	Neutral	Alkaline, 0.05 per cent NaOH	Chromate, 0.17 per cent Na_2CrO_4	Silicate, 0.13 per cent 40°Be.Sil.	
Single metals:									
Aluminum	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{ccc}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{cccc}}$	O.K.	$\frac{\text{O.K.}}{\text{t}}$	ccc
Copper	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{cct}}$	$\frac{\text{O.K.}}{\text{ct}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{ct}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{cc}}$	ccc
Iron	$\frac{\text{c}}{\text{c}}$	$\frac{\text{cc}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{c}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{ccc}}$	cccc
Nickel	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	$\frac{\text{O.K.}}{\text{tt}}$	c
Tin	O.K.	O.K.	O.K.	$\frac{\text{O.K.}}{\text{t}}$	O.K.	$\frac{\text{O.K.}}{\text{cc}}$	O.K.	O.K.	c
Zinc	$\frac{\text{c}}{\text{ccc}}$	$\frac{\text{c}}{\text{ccc}}$	O.K.	$\frac{\text{c}}{\text{ccc}}$	$\frac{\text{cccc}}{\text{cc}}$	$\frac{\text{cccc}}{\text{cc}}$	$\frac{\text{cccc}}{\text{O.K.}}$	$\frac{\text{cccc}}{\text{cc}}$	cccc
Plated metals:									
Copper, tinned E. B.	O.K.	$\frac{\text{O.K.}}{\text{cc}}$	O.K.	O.K.	$\frac{\text{t}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{ccc}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{cc}}$	cccc
Copper, tinned E. T.	O.K.	$\frac{\text{O.K.}}{\text{cc}}$	O.K.	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{cccc}}$	O.K.	$\frac{\text{O.K.}}{\text{c}}$	cccc
Copper Retin	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{ccc}}$	O.K.	$\frac{\text{O.K.}}{\text{cc}}$	O.K.	$\frac{\text{O.K.}}{\text{ccc}}$	O.K.	O.K.	cc
Iron, galvanized	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{ccc}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{ccc}}{\text{cc}}$	$\frac{\text{ccc}}{\text{cc}}$	$\frac{\text{cc}}{\text{c}}$	$\frac{\text{cc}}{\text{cc}}$	cccc
Iron, tinned E. B.	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{c}}$	cccc
Iron, tinned E. T.	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{ccc}}$	$\frac{\text{c}}{\text{c}}$	$\frac{\text{c}}{\text{c}}$	cccc
Al. alloy, Al. plated	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{ccc}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{cccc}}$	O.K.	$\frac{\text{O.K.}}{\text{t}}$	ccc
Alloys:									
Ascoloy	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{c}}$	O.K.	O.K.	O.K.	O.K.	O.K.	$\frac{\text{O.K.}}{\text{tt}}$	ccc
Allegheny Metal	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	O.K.	$\frac{\text{O.K.}}{\text{t}}$	c
Enduro	O.K.	O.K.	O.K.	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{c}}$	O.K.	O.K.	$\frac{\text{O.K.}}{\text{ttt}}$	cc
Monel Metal	$\frac{\text{O.K.}}{\text{t}}$	O.K.	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{c}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{ctt}}$	cc
Nickel silver	$\frac{\text{O.K.}}{\text{ttt}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{cct}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{t}}$	tt
Mn. Al. alloy	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{ccc}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{cc}}$	$\frac{\text{O.K.}}{\text{t}}$	$\frac{\text{O.K.}}{\text{cccc}}$	O.K.	$\frac{\text{O.K.}}{\text{t}}$	ccc

nearly as possible exposure of metal surfaces in practical milk plant operation, such as prevails in steel jackets and vat coils, used both for heating and cooling of the dairy product. This was done by exposing the metal strips successively for two hours each to steam, to cooling water, and to neutral calcium brine. The brine-wet strips were then held overnight and the same treatment was repeated daily for seven consecutive days.

At the conclusion of each experiment the extent of corrosion was determined by recording the loss of weight of the metal strips and noting the appearance of the strips and of the liquids.

Results obtained with refrigerating brines. The results in brine solutions are shown in tables 8 and 9. Table 8 gives the weight losses or gains in milligrams per metal strip exposed. In table 9 is shown the appearance of the metal strips from the standpoint of visible corrosion and tarnish.

Discussion of results shown in tables 8 and 9. A general survey of the results given in table 8 shows markedly greater corrosion and loss in weight in the case of sodium brines, both treated and untreated, than with calcium brines. This difference between sodium and calcium brines was greatest with alkaline brines and least with the neutral brines. On the basis of loss in weight the ratio of corrosion of calcium to sodium brine for all the metals in all the brines excepting the alkaline brines was approximately 1:1.46. In the alkaline brines the ratio rose to 1:2.32. This is in part due to the extraordinary corrosiveness of the aluminum products in alkaline solutions. The total change in weight for all the metals in the different brines used was as follows:

TREATMENT	CALCIUM BRINES	SODIUM BRINES
	mgm.	*mgm.*
Neutral	94 1	114 0
Alkaline	162.2	376.7
Chromate-treated	49.2	71.8
Silicate-treated	85.6	144.5

The above figures show that chromate-treated brines were least corrosive and the alkaline brines most corrosive. It is interesting here to point out that the maintenance of alkalinity in brine by the use of lime, caustic soda, or soda ash has long been recommended in refrigeration practice. Thus, Poste and Donauer (6) advise that in order "to keep corrosion at a minimum the alkalinity must be greater than 0.1 per cent," and Hull (7) makes the following statement: "The ideal brine is one which is as clear as water. A brine kept properly alkaline will keep cleaner and clearer than an acid brine due to the minimized corrosion. Hence, the tremendous practical value in the cost of operation of keeping the brine properly alkaline."

On the other hand, Whitman, Chappell, and Roberts (8) in their report submitted to and approved by the A.S.R.E. Corrosion Committee, show that while slight alkalinity in brine does diminish its corrosive action, high alkalinity increases the pitting tendency of brine. Their recommendations are as follows: "Brines should be maintained very slightly alkaline (pH = 9.5) or just beyond the color change of phenolphthalein. This is the most desirable alkalinity for both iron and zinc in calcium and sodium brines. More acid brines cause increased corrosion while more alkaline ones cause severe pitting."

Although our own experiments were not intended to determine the most desirable brine treatment for corrosion prevention, it is interesting to note that our results with relatively high alkalinity brines appear to indicate that such high alkalinity is not entirely desirable. A few experiments with strips of iron, tinned iron, and copper, showed that the higher alkalinity cleared the brine and that the visible corrosion of the strips appeared to become less general. However, no consistent differences could be observed in weight losses of the iron strips between the neutral and alkaline brines. These tests included brines made slightly acid by passing carbon dioxide through the liquid, neutral brines and brines of the following alkalinities, 0.01, 0.025, 0.050, and 0.1 per cent. As the alkalinity diminished the solution became increasingly rusty red and more rust appeared on the metal strips until in the case of the slightly acid brines the solution showed very heavy red color and precipitate, and the strips were completely covered with rust. This would seem to indicate that the greater the alkalinity of the brine the less the corrosion of the metal. However, as mentioned above, the weight losses differed very little. Thus, in the alkaline brines the iron strips lost in 10 days 15.1, 5.6, and 6.6 mgm., respectively. These brines contained no red precipitate and were water-white, showing only a few dark rust specks in the bottom. Three similar strips immersed in neutral brines lost respectively 13.4, 6.6, and 5.9 mgm., and the brines were all intensely brown and rusty. These results with iron indicate that although the alkalinity clarified the brine it did not retard nor diminish corrosion of the metal. In fact, it tended to intensify localized corrosion as was also shown by Whitman, Chappell, and Roberts (8), and such localized corrosion or pitting in the case of highly alkaline brines may well become more damaging and objectionable than the more generalized corrosion in the case of only slightly alkaline brines.

In the case of the tin strips the alkaline brines caused distinctly greater corrosion than the neutral brines. Even an alkalinity of 0.025 per cent produced marked corrosion on the tin and the corrosion in the presence of 0.05, 0.10, and 0.13 per cent was proportionately greater. All alkaline brines appeared to also attack the copper.

Another mechanically objectionable feature of the use of high-alkalinity brines is that in the case of calcium brine which has been made alkaline with sodium hydroxide, a fine precipitate of calcium hydroxide gradually accumulates in the circulating system. This precipitate tends to insulate the pipes and to interfere with the proper functioning of valves and pumps. Theoretically the formation of this objectionable precipitate is readily prevented by adding the caustic soda to the brine in highly dilute solution and very slowly, so as to avoid localized supersaturation of calcium hydroxide in the brine. In practical operation, however, the observation of these precautions is accompanied by serious inconvenience and, in fact, these precautions are not observed with sufficient care to avoid precipitation of calcium hydroxide and the gradual accumulation in the brine system of an objectionable deposit.

In results given in tables 8 and 9 the metals that showed the greatest resistance to all the brines were nickel and Allegheny

Metal. These two metals yielded no visible corrosion on the strips and no precipitate in the liquid. Their changes in weight also were very low and in many instances there was no loss nor gain whatever. All the other metals varied greatly in their resistance to the various types of brines. In some instances they resisted corrosion completely, while in others they were severely attacked. It appears advisable, therefore to discuss these results according to type of brine used.

Neutral calcium brine. In this brine, nickel, Allegheny Metal, Enduro, and Monel Metal suffered practically no attack. The loss in weight was also very slight with nickel silver and with the aluminum products, but nickel silver tarnished heavily and the aluminum products showed considerable visible corrosion. Likewise tin and tinned copper were free from visible corrosion, but suffered appreciable loss in weight. All the other metals manifested severe corrosion as evidenced both by loss in weight and by marked visible attack on the metal strips, and, in some cases, by a heavy precipitate in the brine. Zinc, iron, and copper showed the least resistance.

Neutral sodium brine. The corrosion in neutral sodium brine was considerably greater than in the calcium brine, but here again nickel and Allegheny Metal were free from visible corrosion and their weight losses were small. Enduro rusted slightly along the edges of the strips but its loss in weight was slight. Tin, Monel Metal, and the aluminum products showed only slightly greater attack. The rest of the metals suffered considerably more corrosion, iron, copper, zinc, and galvanized iron being the least resistant.

Similar high resistance of Allegheny Metal and Enduro in saturated calcium and sodium brines was also reported by Quam (5), who states that "the high chromium steels showed no change in surface appearances but low chromium steel developed a few black corrosion spots and showed much greater loss." The high chromium steels above referred to consisted of Allegheny Metal and ordinary chromium steel containing 17.5 to 18.5 per cent Cr., which conforms approximately to the chromium content of Enduro.

Alkaline calcium brine. In general the metals showed lower resistance to attack in alkaline calcium brine than in neutral calcium brine. This does not apply, however, to nickel, Allegheny Metal, Enduro, Monel Metal, nickel silver, and Ascoloy. The aluminum products were very severely attacked and the tinned products also suffered material corrosion. The iron strips appeared to be more intensely corroded but the liquid contained less rust in suspension. Copper also suffered severely and produced a blue color in the brine.

Alkaline sodium brine. The alkaline brine here too caused much greater corrosion than the neutral brine. This was particularly true for the aluminum products, zinc, galvanized iron, and most of the tinned products. The loss in weight here averaged nearly three times greater than in the neutral brine. Nickel, Allegheny Metal, Enduro, Ascoloy, Monel Metal, and nickel silver, however, resisted corrosion fully as well as, and in some cases even better, than in the neutral brine.

Chromate-treated calcium brine. The addition of a small amount of chromate to the neutral calcium brine very greatly increased the resistance to corrosion of most of the metals, reducing the average loss in weight of all the metals to less than one-half of that in plain neutral calcium brine. The corrosion-retarding effect was particularly marked in the case of the aluminum products and the tinned metals. The metals that suffered appreciable loss in weight here were zinc, galvanized iron, iron, and copper.

Chromate-treated sodium brine. The corrosion-retarding effect of chromate in sodium brines was similar to that in calcium brine. It is noteworthy here too that the chromate treatment did not appreciably increase the resistance to corrosion of iron and galvanized iron.

In the January, 1926, Report of the A.S.R.E. Corrosion Committee, Whitman, Chappell, and Roberts (8) recommend 0.17 per cent sodium di-chromate as a suitable concentration for chromate concentration in very slightly alkaline calcium brine. This same chromate concentration was provided in both the calcium and the sodium brines used in the present experiment. In the

January, 1927, report of the above committee, Russell, Roberts, and Chappell (9), as the result of later research, recommend 100 pounds of sodium di-chromate per 1000 cubic feet of calcium brine which, according to their graph, is equal to about 0.16 per cent sodium di-chromate, while for sodium brine they recommend the equivalent of twice of this chromate concentration, or 0.32 per cent sodium di-chromate, for a closed brine system. These investigators suggest that either sodium di-chromate or sodium chromate may be used, but they further report that the chromate is the more effective of the two and that the cheaper commercial di-chromate may be changed to the neutral chromate by the addition of the proper amount of caustic soda. They recommend 35 pounds of caustic soda per 100 pounds of the di-chromate. Their tests were confined to iron, steel, and galvanized steel. In a still later report of the Corrosion Committee, Roberts, Forrest and Russell (10) state that "it has been found in laboratory as well as in plant tests that the best protection (for iron, steel, and galvanized steel) is obtained when the brine is made slightly alkaline after the addition of the di-chromate."

Silicate-treated calcium brine. The addition of sodium silicate to calcium brine had but slight effect on the resistance to corrosion of the various metals. Moreover, the silicate-treated brine contained a very considerable precipitate which renders the use of silicate in calcium brine undesirable.

Silicate-treated sodium brine. Except in the case of zinc and galvanized iron the addition of sodium silicate to neutral sodium brine resulted in a considerable improvement in the resistance of most of the metals. The above two metals, however, showed a marked increase in corrosion and loss in weight over that in untreated neutral sodium brine.

Russell, Roberts, and Chappell (9) state that "although sodium silicate is an excellent corrosion preventive for iron and steel in fresh water, it has been found ineffective in brines. Tests, made both in the laboratory and in plants, using 40° Be. silicate, have demonstrated that little protection either to galvanizing or to bare metal results from the use of silicate."

Successive treatment with steam, water, brine, and air. In commercial plant operation the heating and cooling of the milk product is usually accomplished by the successive use of steam, water, and brine, and the brine-wet metals are then left exposed to the air until the equipment is again needed. In order to study the effect of such exposure of metals experimentally, the metal strips were successively steamed in an autoclave, completely immersed in cooling water and then in cold brine. Each exposure continued for two hours. The brine-wet strips were then held in the air overnight and the same treatment was repeated daily for seven consecutive days.

The results, tables 8 and 9, show corrosion of all the metals used. From the standpoint of visible corrosion nickel, tin, and Allegheny Metal were only slightly affected, and their weight losses were very small. Iron and galvanized iron on the other hand suffered such outstandingly great weight losses as to condemn their use for such purposes. These results explain the rapid and profuse rusting and short life of steel-jacketed tanks used for both heating and cooling of milk and cream. The aluminum products also lost heavily in weight and all the metals except nickel, tin, and Allegheny Metal suffered severe visible corrosion. In a separate experiment in which the metal strips were exposed to steam alone the weight changes were much smaller, and in most cases negligible. These results, together with those obtained from the single brine treatment, show convincingly that this combination of heating and brine-cooling is far more destructive to the metals than the use of steam or brine alone.

SUMMARY AND CONCLUSIONS

The resistance to corrosion of 19 different metals, plated metals, and metallic alloys in solutions of washing powders, chemical sterilizers, and refrigerating brines was studied. The metals consisted of aluminum, manganese aluminum alloy and aluminum plated alloy; copper, nickel, Monel Metal, and nickel silver; tin, tinned copper, and tinned iron; iron, zinc, and galvanized iron; and ordinary chromium steels in the form of Ascoloy and Enduro, and a chromium-nickel steel in the form of Allegheny Metal. The metals used consisted of strips which were immersed in the solutions studied to the extent of one-half of the length of the strips. At the conclusion of each series of tests the weight losses and appearance of the strips and the color and precipitate of the liquids were noted. The results of these tests were briefly as follows:

A. In washing solutions

1. The washing solutions used were 0.5 per cent solutions of sodium hydroxide, special alkali, sodium carbonate, Wyandotte Cleaner and Cleanser, tri-sodium phosphate, and tri-sodium phosphate containing 0.025 per cent chromate of soda. In an additional investigation the corrosion-protective effect of silicate and chromate of soda on aluminum products was studied.

2. The aluminum products suffered by far the most intense corrosion in all the washing solutions used, the weight losses of the three aluminum products accounting for over two-thirds of the total weight losses of all the metals.

3. The tinned copper and tinned iron products ranked next in intensity of corrosion. Their resistance, however, was much greater than that of the aluminum products, the total weight losses of the five tin-coated products being only one-seventh of those of the entire 19 metals. Tin, itself, showed varying corrosion, but as a whole it resisted attack better than the tin-coated products.

4. Of the remaining metals the chromium-nickel steel, Allegheny Metal, and the ordinary chromium steels, Ascoloy and Enduro, proved most resistant, in fact, these three chromium steel alloys were practically immune to the action of the washing solutions. Nickel and Monel Metal also showed only negligible weight losses and withstood visible corrosion and tarnishing well. Nickel silver, while not suffering appreciable weight losses, tarnished considerably in some of the washing solutions, while the corrosion of copper, iron, galvanized iron, and zinc was decidedly greater but not as severe as that of the tin-plated products.

5. In the case of the aluminum products, corrosion in alkali washing solutions was avoided by treating sodium carbonate with a very small amount of silicate of soda. Thus, a 0.5 per cent

solution of sodium carbonate containing 0.05 per cent of sodium silicate eliminated the weight losses of the aluminum products in this washing solution entirely.

6. In a similar manner the corrosion of tin-plated copper and tin-plated iron in alkali washing solutions may be reduced to negligible figures by chromate treatment of tri-sodium phosphate. This was accomplished by the use of 0.5 per cent solutions of tri-sodium phosphate containing 0.025 per cent chromate of soda. These facts are important in that they assist in solving the difficult problem of preserving the tin coating in milk and cream shipping cans, pasteurizing vats, and other tinned surfaces in milk plant equipment. They further emphasize the short life of the tinned surfaces when caustic and other strongly alkaline washing solutions are used.

B. In chemical sterilizers

1. The chemical sterilizers used were solutions of sodium hypochlorite, Diversol, and chloramine-T.

2. In solutions made up with approximately equal content of available chlorine, the sodium hypochlorite was by far the most corrosive of the three chemical sterilizers used.

3. Diversol, on account of its alkaline properties, proved very destructive to aluminum and is, therefore, not suitable for use on aluminum equipment. However, it was the least corrosive sterilizer in the case of all the other metals and its effect on the tin-plated products was relatively slight.

4. Chloramine-T also showed far less intense action on tinned products than sodium hypochlorite but its corrosive effect was somewhat greater than that of Diversol.

5. Allegheny Metal and Enduro showed no visible corrosion in any of the chemical sterilizers, and the attack on Ascoloy, nickel, Monel Metal, and nickel silver was but slight.

6. In neutralizing lime the aluminum products were very severely affected and the presence of oxidation products was evident. Zinc and galvanized iron also suffered considerable corrosion and weight losses. The attack on the remaining metals was slight.

C. In refrigerating brines

1. Both neutral sodium chloride brine and neutral calcium chloride brine were used. Portions of these two brines were also made alkaline with caustic soda and still other portions were treated with sodium chromate and sodium silicate, respectively. In addition a series of metal strips was exposed to successive treatment with steam, water, brine, and air.

2. Nickel and Allegheny Metal resisted the corrosion practically completely. In only two cases did these metals show any attack whatever and then only to a slight extent. This was in the case of the combined treatment with steam, water, brine, and air, and in the silicate-treated sodium brine. But even here these two metals were more resistant than any of the others.

3. Nickel and Allegheny Metal were closely followed by Enduro, Ascoloy, Monel Metal, nickel silver, and tin, all of which showed only slight attack in most of the brines.

4. Copper and the tinned iron and tinned copper products showed considerable corrosion. This was especially true of the tinned products in the alkaline brines.

5. The aluminum products withstood corrosion to a marked degree in all brines except in the alkaline brines in which they were severely attacked.

6. Iron, galvanized iron, and zinc suffered heavy weight losses in all the brines and showed very poor resistance to corrosion.

7. With reference to the comparative corrosiveness of the individual brines, our results indicate that, in general, the action of the sodium brines was more severe then that of the calcium brines; that chromate treatment affords the best retardation of corrosion, and that in the case of some of the metals, an alkalinity of 0.05 per cent intensified corrosion. This was particularly true of the aluminum, tin, and zinc products.

ACKNOWLEDGMENTS

We are greatly indebted to the Allegheny Steel Company, the Aluminum Company of America, D. H. Burrell & Company, J. G. Cherry Company, the Creamery Package Manufacturing

Company, The International Nickel Company, and the Jensen Creamery Machinery Company, for materials and data generously placed at our disposition.

REFERENCES

(1) Hunziker, O. F., Cordes, W. A., and Nissen, B. H. 1929 Metals in dairy equipment. Metallic corrosion in milk products and its effect on flavor. Jour. Dairy Sci., xii, no. 2.

(2) North, C. E. 1927 Report on chemical and electrical tests on metals. Report by The Creamery Package Manufacturing Company, Chicago, Ill.

(3) Seligman, R. 1923 Milk and metals. Proc. World's Dairy Congress, ii, 1211.

(4) Churchill, H. V. 1927 Information by correspondence with The Aluminum Company of America.

(5) Quam, G. N. 1927 Comparison of the corrosion resistance of three kinds of stainless steels. Report by J. G. Cherry Company, Cedar Rapids, Iowa.

(6) Poste, E. P., and Donauer, M. 1923 The corrosion of metals by refrigeration brine. The Milk Dealer, February issue.

(7) Hull, B. S. 1923 Chemistry of the brine tank. Ice and refrigeration, lxiv, 175.

(8) Whitman, W. S., Chappell, E. L., and Roberts, J. K. 1925 Corrosion in the Refrigerating Industry. Report to the Corrosion Committee of The American Society of Refrigerating Engineers. Refrigerating Engineering, xii, 158.

(9) Russell, R. P., Roberts, J. K., and Chappell, E. L. 1927 Corrosion in the Refrigerating Industry. Report to the Corrosion Committee of The American Society of Refrigerating Engineers. Refrigerating Engineering, xiii, 209.

(10) Roberts, J. K., Forrest, H. O., and Russell, R. P. 1927 Corrosion in the Refrigerating Industry. Report to the Corrosion Committee of The American Society of Refrigerating Engineers. Refrigerating Engineering, xiv, 173.

A DEVICE FOR REDUCING AN ICE CREAM MIX TO ITS BASIC VISCOSITY*

RANDALL WHITAKER

Department of Dairy Industry, Cornell University, Ithaca, New York

In the manufacture of commercial ice cream, the mix is usually aged or ripened for one or two days to obtain the beneficial effects which are believed to be associated with the higher viscosity resulting from this practice. The work of Leighton and Williams[1] indicates that the increased viscosity of the unagitated aged mix, called the apparent viscosity, is due to the combined action of the concentration and composition of the mix and a mechanical structure which is formed during the aging process. These workers subjected ripened ice cream mixes to the agitation caused (1) by the dashers of an ice cream freezer, (2) by a small laboratory stirrer, and (3) by vigorous shaking; and found that the mechanical structure was destroyed. The viscosity decreased with agitation to a constant value, which these workers called the basic viscosity of the mix. They showed that this basic viscosity depends upon the concentration and composition of the mix, and is of more significance than the apparent viscosity which is so rapidly destroyed in the freezer. Experimental studies conducted in this department have also shown that basic viscosity values are more reliable and reproducible than those expressed as apparent viscosity.

APPARATUS FOR DETERMINING BASIC VISCOSITY

A very simple, convenient, and efficient device for reducing an ice cream mix to its basic viscosity has been developed during the course of these experiments. A cross section diagram of the apparatus is shown. A motor driven stirrer made of wire screening revolves in a glass cylinder containing the mix. In using the

---

* Received for publication December 13, 1928. Supported by a grant from the American Dry Milk Institute.

[1] Jour. Phys. Chem., 1927, xxxi, 596.

apparatus the cylinder is first completely filled with mix, and the rubber stopper carrying the shaft of the stirrer is then carefully inserted in the cylinder in such a manner as to insure the removal of all air bubbles. As the stopper is pressed down into place the excess mix is forced up into the overflow tube provided. Should the stopper loosen slightly due to the vibration, or should a leak occur at the bearing, additional mix will readily flow down from the funnel shaped overflow to replace that which was lost, and prevent the entrance of air. A speed of 450 to 500 r.p.m. for a period of twenty minutes is sufficient to reduce the mix to its basic viscosity when measured with a Mojonnier-Doolittle viscom-

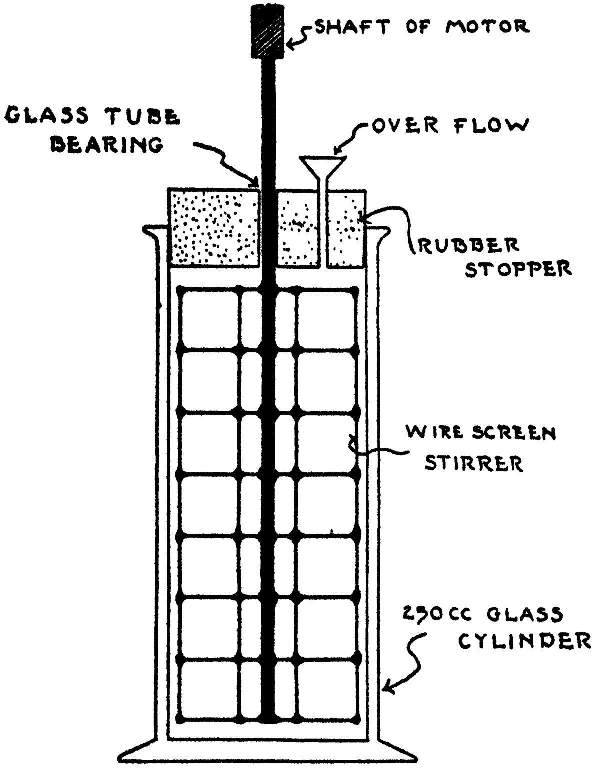


FIG. 1. DEVICE FOR REDUCING ICE CREAM MIX TO ITS BASIC VISCOSITY

eter. The cylinder may be immersed in a water bath of any desired temperature. A temperature of 0°C. was found to be convenient. This was easily maintained by an ice and water mixture. The viscosity must be determined immediately after the stirring has been completed.

FACTORS AFFECTING THE EFFICIENCY OF THE APPARATUS

In the construction and operation of this device the time of stirring, the speed of the agitator, the size of the wire screening, and temperature of the mix are variable factors which would appear to affect its efficiency in reducing the viscosity of an ice cream mix to a reproducible minimum. All determinations were made at 0°C. to duplicate as closely as possible conditions found in the freezer. Tests were made in which the time of stirring was varied until it was evident that, with a speed of 450 to 500 r.p.m., twenty minutes allowed ample time for the viscosity of the mix to reach a minimum. Tests were also conducted with stirrers constructed of different sized wire screening. The wires of the screens were $\frac{1}{4}$ inch, $\frac{3}{8}$ inch, and $\frac{1}{2}$ inch apart respectively. The three agitators all reduced the mix to its basic viscosity when the device was operated at the prescribed rate of speed for twenty minutes, although the destruction of the apparent viscosity was effected a little more rapidly with the stirrers constructed of the smaller mesh screening.

THE RELATION OF THE HYDROGEN-ION CONCENTRATION TO THE TEXTURE OF EMMENTHAL OR SWISS CHEESE*

PAUL D. WATSON

Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture

INTRODUCTION

The object of the work described herein was the determination of the relation of the march of hydrogen-ion concentration in Emmenthal or Swiss cheese to the texture. In this research differences in the proteolysis and the moisture of the cheese were correlated with the above factors. For the purpose of comparative study variations were produced by the use of two starters, the *ya* starter, a bulgaricus organism with a mycoderm, and the *39a* starter, an old strain of *Lactobacillus bulgaricus.*

Orla-Jensen (1) has studied the influence of the degree of acidity upon the consistency and ripening process of Emmenthal cheese and this work will be referred to later. Boekhout and Ott de Vries (2) found that an excessive amount of free lactic acid was the direct cause of brittle texture in the Edam cheese. Van Dam's (3) researches on Edam cheese indicated that the texture was a phenomenon of colloidal chemistry, and that an excess of hydrogen ions also produced a hard and brittle texture. He also found that the progress of ripening was dependent upon the proportion of acid present. Leitch (4) who worked with English Cheddar cheese stated that the degree of acidification of the curd was the factor which exercised the greatest control over the texture of the cured cheese. In an investigation of Cheddar cheese Van Slyke and Hart (5) found that excess acid produced a dry, harsh, and hard texture and that cheese having the most moisture generally contained the largest amounts of soluble nitrogen. Van Slyke

---

* Received for publication December 7, 1929. Presented before the Division of Agricultural and Food Chemistry at the Seventy-sixth Meeting of the American Chemical Society, Swampscott, Mass., September 10 to 14, 1928.

(6) also found that excessive moisture produced a texture of undesirable softness, while deficient moisture favored a dry and crumbly texture. It appears, then, that moisture and acidity exert an influence upon the texture and ripening of cheese, and this has been brought out in the present study.

Although considerable interest has been attracted in recent years to the significance of the hydrogen-ion concentration in biochemical phenomena, it is thought that control of this factor does not always imply entire control of the cheese-making process. Moreover, the variability which is ever present in biochemical processes and the fact that only very limited correlations can be carried out in one laboratory prevent synchronous studies from having the completeness desired.

EXPERIMENTAL METHOD

Each pair of experimental cheese was made upon a separate day under carefully controlled conditions which approximated commercial practice. Precautions were taken to have identical milk and the same quantities of starter, eye culture, and rennet in each cheese of a pair. The deviation in the making of the two cheese consisted in the addition of the *ga* starter to one and the *39a* starter to the other, 0.25 per cent being added in each case. The starters were grown in skim milk for 18 hours at a temperature of 42°C.; the *ga* starter averaged 1.9 per cent of lactic acid and the *39a* starter averaged 1.3 per cent. Each cheese weighed between 50 and 60 pounds when green.

The last six pairs of cheese in table 1 were made from milk which had been ripened by holding over night at a temperature of approximately 16°C. This ripening was irrelevant to these experiments. The cooking temperature of all the cheese was 53°C., with the exception of three pairs in the preliminary experiments which varied one degree from this figure.

METHODS OF STUDY

Determinations of pH and lactose content were made while the cheese were upon the press during the first 24 hours after dipping. Comparative studies were also made of the pH, proteolysis, mois-

TABLE 1

Comparison of cheese made with ga and 39a starters with reference to the pH, lactose, proteolysis, moisture and freezing points

CHEESE NUMBER	VARIATION IN STARTER		pH AT			PROTEOLYSIS				PER CENT MOISTURE	FREEZING POINT	PER CENT MOISTURE PER DEGREE FREEZING POINT LOWERING	TEXTURE AT 18°C.
							In per cent total nitrogen		In per cent of soluble N				
	0 25 per cent starter	Per cent acidity	3 hours	21 hours	Maturity	Per cent of lactose at 21 hours	Soluble N	Amino-N	Amino-N				
											°C.		
312	ga	1 8	6 14	5.13	5.59	0 00	21 0	10.8	51 2	35.1	−7.7	4 56	10
312-1	39a	1 3	6 31	5 48	5.68	0 81	19 6	8 1	41 4	35.1	−8.6	4 08	30
314	ga	1 7	6.06	5 09	5 60	0 00	23 5	13 7	58 4	34 1	−7.0	4 87	8
314-1	39a	1 2	6 31	5.50	5 75	0 74	24 5	10.7	43 8	35 1	−7 2	4.88	44
315	ga	1 6	6 21	5 05	5 50	0 00	30 2	14 7	48 7	34 2	−7.0	4.89†	18
315-1	39a	1.3	6 35	5 55	5 80	0.91	27.0	11 1	41 3	34.8	−6·6	5.27	52
x 372	ga	2.1	5 85	5 01	5 60		32.8	16 8	51.3	34 2	−7 5	4.56†	18
372-1	39a	1.4	6.14	5.25	5.75		33 4	15.0	44.8	34.7	−7 0	4 96	76
x 373	ga	1.9	5 80		5 65		27 7	17.1	61 7	33 3	−8.1	4 11†	15
373-1	39a	1 4	6.04		5 58		24.0	12 6	52.5	34 3	−7.6	4.51	61
x 428-1	ga	2 0	5.89	5.00	5 54		23 7	16.1	67 9	35 0	−7.4	4 73†	13
428	39a	1.3	6.19	5.29	5.39		24.2	11 4	47.3	34 4	−6 6	5 21	41
x 429-1	ga	1.9	5.92	5 03*	5 53		23.9	14.4	60 0	33 3	−8 6	3 87†	5
429	39a	1.3	6.18	5.35*	5.50		25.0	12 7	50 8	33.5	−8 0	4 19	9
x 430-1	ga	2 0	5.83	4.98	5.58		34 7	22.6	65.2	33.5	−9 0	3.72†	6
430	39a	1.5	6 04	5.19	5 47		26.6	12.8	48 0	34.5	−7.8	4 42	30
x 431-1	ga	1.9	5.99	5 01	5.65		23 1	15 8	68 7	32 3	−8.7	3 71†	5
431	39a	1 3	6 29	5.38	5.48		27 2	12 8	47.1	32.9	−7.5	4 39	33

Pairs marked x were made from ripened milk.

* pH at 23 hours.

† The ga cheese generally had a smaller percentage of moisture per degree of freezing point lowering than the 39a cheese, which indicated a greater percentage of "bound" water in the latter.

ture, freezing point, and texture, when the cheese was 3.5 to 5 months old. These data are tabulated in table 1. Figures 3, 4, 5, and 6 are based on the results shown in the table.

The quinhydrone electrode was used for the pH determinations. The two electrodes used for the whey and plug samples are shown in figure 1. This method has been previously described by the author (7).

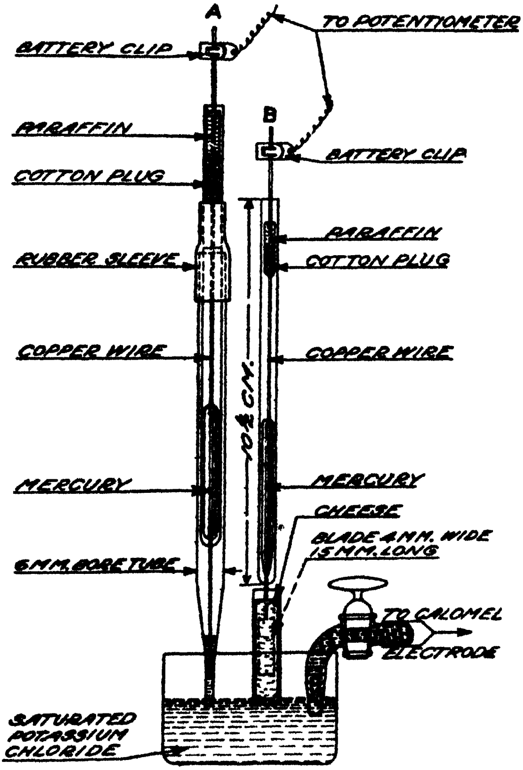


FIG. 1. TYPES OF THE QUINHYDRONE ELECTRODE USED FOR THE MEASUREMENT OF HYDROGEN-ION CONCENTRATION

A, Cullen and Biilmann capillary electrode; *B*, Knudsen cheese electrode

The lactose content was determined by the colorimetric picric acid method of Bierman and Doan (8), which is a special method for dairy products.

The amount of total and soluble nitrogen in the first three pairs of cheese in table 1 was found by the direct nesslerization micro-Kjeldahl methods of Golub (9) and of Koch and McMeekin (10); the digestion was carried out according to the former, and the

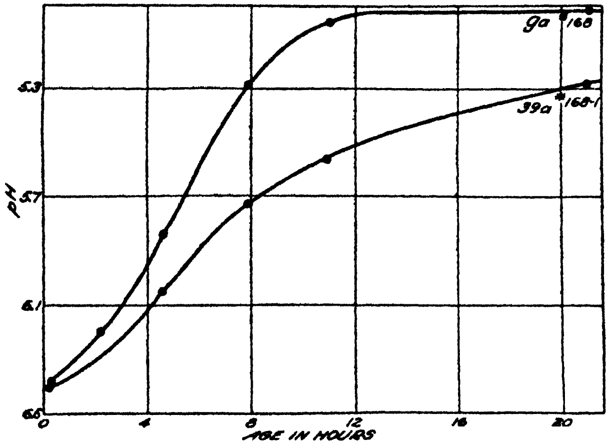


FIG. 2. CURVES SHOWING THE VARIATION OF pH DURING A 20-HOUR PERIOD IN A PAIR OF CHEESE MADE WITH THE TWO STARTERS

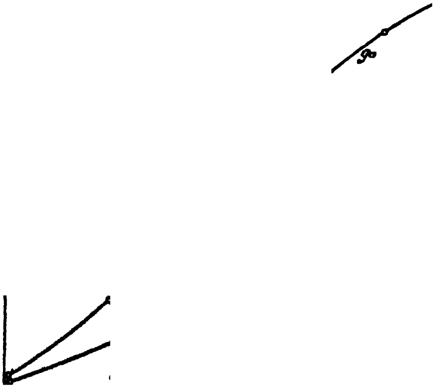


FIG. 3. CURVES SHOWING THE VARIATION OF pH DURING A 10-HOUR PERIOD IN A PAIR OF CHEESE MADE WITH THE TWO STARTERS

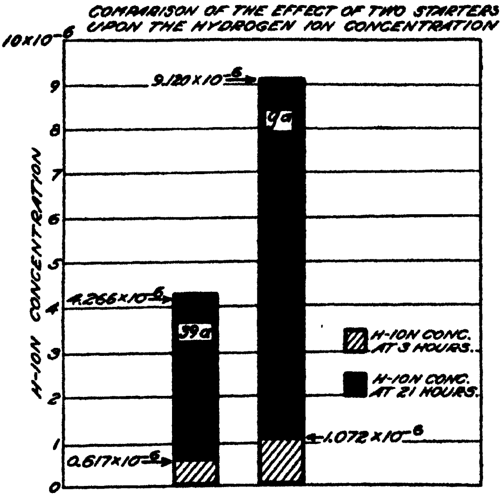


FIG. 4. COMPARISON OF THE EFFECT OF TWO STARTERS UPON THE HYDROGEN-ION CONCENTRATION

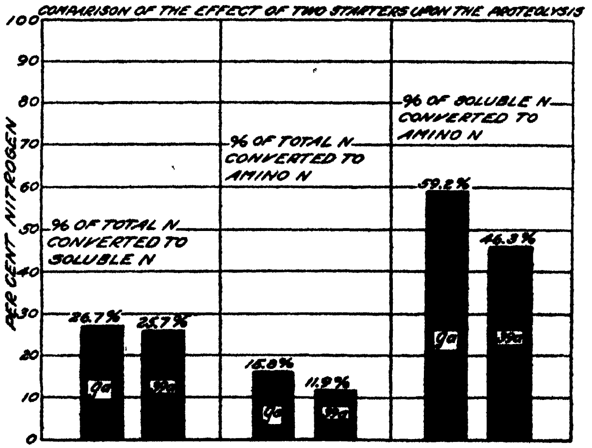


FIG. 5. COMPARISON OF THE EFFECT OF TWO STARTERS UPON THE PROTEOLYSIS

nesslerization according to the latter. The amount of total and soluble nitrogen of the remaining pairs was determined by the gasometric micro-Kjeldahl method of Van Slyke (11).

The water soluble nitrogen content was measured by extracting a 25 gram sample with water at about 50° to 55°C. and making it up to 500 cc. volume. The solution was then filtered and aliquot samples taken for analysis. In the first two pairs of cheese the samples were extracted after the method of Van Slyke and Hart (12) and the remainder after that of Teichert (13).

The amino nitrogen content of the water soluble extract was measured in the Van Slyke (14) apparatus for the determination of aliphatic amino nitrogen. The small quantity of ammonia in the extract was not removed, as Van Slyke states that good comparative results can be obtained without this precaution if conditions are kept constant.

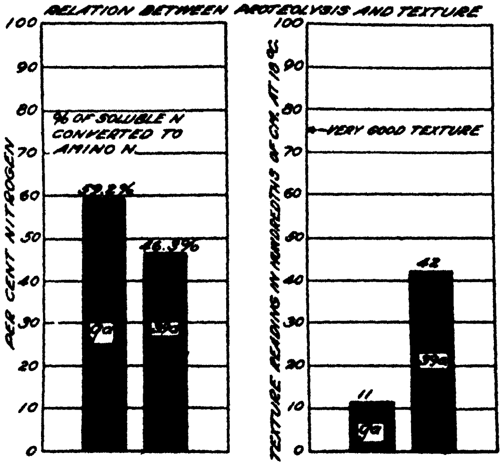


FIG. 6. RELATION BETWEEN PROTEOLYSIS AND TEXTURE

The values obtained by these methods in the various nitrogen analyses of the cheese are comparable to those found by other investigators (15).

The moisture content of the cheese was determined upon 4 to 5 gram samples mixed with dry sand by using the vacuum oven method at 98° to 100°C. for a period of 7 hours.

The freezing point of the cheese was measured electrometrically by means of thermocouples according to the method previously described by the author (16).

The texture of the cheese was measured by means of a penetrometer. The number of hundredths of a centimeter that a needle weighted with 200 grams penetrated the cheese in a period of 15 seconds at a certain temperature was recorded as the texture. The texture readings were generally taken from texture curves for the temperature of 18°C.

The samples for the various tests were taken from as near the center of each cheese as possible, and the determinations were made in duplicate except in the case of cryoscopic measurements.

PRELIMINARY EXPERIMENTS

Some preliminary experiments were made which served as a basis for the later procedure. The results of these experiments are not tabulated herewith, but are merely summarized. Fourteen pairs of cheese were made in the manner described above with exceptions of only minor importance, and the following results were arrived at:

It was found the the hydrogen-ion concentration in the *ga* cheese at 3 hours and 21 hours after dipping was, respectively, 1.6 times (average of 12 pairs) and 1.9 times (average of 10 pairs) that of the *39a* cheese.

The lactose content as determined for three pairs of cheese after 21 hours, and calculated upon the wet basis, was 0.5, 0.7, and 0.8 per cent for the *39a* cheese as compared with none for the *ga* cheese. Tests made upon a *39a* cheese indicated that only a trace of lactose was present after 45 hours.

The amino nitrogen content of the cheese itself, not the water soluble extract, was determined for three pairs and the excess present in the *ga* cheese over that in the *39a* cheese was calculated upon the dry basis to be 55, 47, and 23 per cent.

The texture of 11 pairs was measured and in all cases the *39a* cheese was superior to the *ga* cheese, the average texture value of the former proving to be 2.8 times that of the latter.

The moisture of 10 pairs was determined at maturity, and it was found with one exception that the moisture in the *39a* cheese exceeded that in the *ga* cheese, the average moisture content being 1.7 per cent greater.

The results briefly outlined above serve to corroborate those obtained subsequently in the nine experiments which are presented in table 1. These later data are more nearly complete and are discussed separately below under several heads.

Hydrogen-ion concentration

The concentration of hydrogen ions in cheese is of essential importance: firstly, in its effect upon the bacteria and enzymes; and secondly, in its effect upon the physico-chemical condition of the proteins. Clark (17) has stated that hydrogen-ion determinations represent the effective relative pressures of the hydrogen ions, and that in one sense an insuperable difficulty is introduced in the rational consideration of cheese, because these effective pressures are influenced by the presence of other ions of high charge and effective ionic radius.

The fermentation of the lactose apparently reaches completion at about pH 5.0, the high buffering value of the curd permitting its continuation until the lactose is entirely consumed, whereupon the growth of the organisms is largely inhibited for lack of sugar which supplies the carbon necessary for their sustenance. The rate at which the fermentation proceeds will depend largely on the number and vigor of the lactic bacilli. Furthermore, it is known that the activity of the various enzymes responsible for the subsequent ripening is influenced by the degree of acidity which is developed in the curd, the activity of the rennet enzyme being favored by acid, while that of the other proteolytic enzymes is retarded (1), (18).

Due to the presence in the green cheese of a large amount of buffers, such as the insoluble calcium salts, in comparison with the small quantity of lactose, the lactic acid formed is neutralized in great part, and free lactic acid is present only in restricted amounts as indicated by the hydrogen-ion concentration determinations. Van Slyke and Bosworth (19) have shown that as a result of the action of the lactic acid upon the calcium paracaseinate of the curd, lime is abstracted from the casein and it becomes soluble in a 5 per cent salt solution. According to Orla-Jensen (1) this property is lost in the presence of an excess of acid and, therefore, the acidity of the curd has an important influence upon the consistency of the cheese for the following reason: When a slightly acid curd is placed in brine in the salting process, it will swell as if tending to dissolve, becoming elastic and semi-transparent, while an excessively acid curd will remain brittle for a long period.

The divergence in pH which occurred during the first 20 hours in the cheese made with the *ga* starter and the *39a* starter is shown in figure 2. These curves show the results obtained from a pair of cheese in the preliminary experiments, and are typical of those which were generally obtained. In figure 3 are shown similar curves plotted over a 10-hour period for one of the pairs in table 1.

The average hydrogen-ion concentration for the two groups of cheese after 3 hours and 21 hours is shown in figure 4. It is seen that the average hydrogen-ion concentration of the *ga* cheese is 1.7 times that of the *39a* cheese after 3 hours and 2.1 times after 21 hours. It was found that the lactose in the *ga* cheese had disappeared after 21 hours, but that in the *39a* cheese it ranged from 0.7 to 0.9 per cent. As is to be expected, the hydrogen-ion concentration of the cheese was inversely proportional to the residual lactose.

A number of studies which have been carried on with cheese made with both starters indicated that the pH of the two cheese, after a few days, tended to approach the same value in the region of pH 5.1 to 5.2. The pH of the two cheese then gradually increased and at the same time deviated again until maturity when it ranged from 5.4 to 5.8. The gradual increase in pH is presumably due to the destruction of the lactic acid and the liberation of the alkaline products of protein decomposition. The pH value of the cheese at maturity appeared to have little significance in regard to the other factors studied. The march of hydrogen-ion concentration in Swiss cheese is comparable to that found in Edam cheese by Van Dam (3).

Proteolysis

The degree of proteolysis or disintegration of the insoluble proteins occurring in the ripening of cheese was studied by determination of the percentage of total nitrogen changed into the water-soluble form and the proportion of the latter converted to amino nitrogen. The data obtained served as a general measure of the extent and intensity of the proteolytic cleavage.

The difference in the proteolysis of the two cheese is shown in figure 5. It will be noted that the total nitrogen converted into soluble form averaged 26.7 per cent for the *ga* cheese as compared with 25.7 per cent for the *39a* cheese. It will be seen that the total nitrogen changed to amino nitrogen averaged 15.8 per cent for the *ga* cheese as against 11.9 per cent for the *39a* cheese. Furthermore, it is shown that the soluble nitrogen converted to the amino form averaged 59.2 per cent for the *ga* cheese as compared with 46.3 per cent for the *39a* cheese.

The data indicate further that the extent of the proteolysis as shown by the soluble nitrogen in percentage of total nitrogen was not markedly different in the two cheese, but that the intensity or thoroughness of the proteolysis as shown by the amino nitrogen when expressed in percentage of soluble nitrogen was much greater in the case of the *ga* cheese.

Texture

When the texture of the two cheese was compared, the softer cheese, or those with the higher penetrometer readings, were regarded as superior to the tougher cheese or those with the lower penetrometer readings. The word texture, as often used in relation to cheese, refers to firmness or consistency of body as well as to the quality of compactnesss. The penetrometer readings are not a measure of all the factors responsible for the texture of a cheese, but they have a direct relationship to them and afford a convenient means of numerical comparison. It is at once apparent from figure 6 that the *39a* cheese were far superior in texture to the *ga* cheese, the average penetrometer readings of the former being almost 4 times as great as those of the latter. In each pair the *39a* cheese invariably possessed the better texture.

Moisture

The data show that the *39a* cheese generally had a little higher percentage of moisture than the *ga* cheese, the average difference being 0.5 per cent. Since this difference is not very marked, it was thought that there might be variations in the state in which the water was held in the cheese, that is, in the proportion of the "free" to the "bound" water. The water which is incapable of being frozen may be regarded as "bound" water and the remainder as "free" or interstitial water (20).

The theoretical freezing point of a cheese just after it is dipped is approximately that of the whey (−0.55°C.). Since the moisture content of a cheese does not decrease markedly with the ripening but since a considerable freezing-point lowering occurs, it was indicated that water must be removed from its rôle as solvent, probably by chemical combination with the proteins of the cheese. It was apparent, therefore, that cryoscopic data might be of service in this differentiation of the state of the moisture in cheese, and freezing point determinations were made upon each.

An examination of the data in table 1 shows that the *ga* cheese generally had a lower percentage of moisture and, as was to be expected, a greater depression of the freezing point than the *39a* cheese. However, if the percentage of moisture per degree of freezing point lowering for each cheese is calculated, it is apparent that with one exception this value is greater for each of the *39a* cheese, a fact which indicates a greater percentage of "bound" water in the *39a* than in the *ga* cheese. This follows from the assumption that when there is more water per degree of freezing point lowering in one cheese than in another, there is then more of the water in the former incapable of being frozen, and therefore bound by the protein. These data were checked by means of another type of experiment upon a pair of *ga* and *39a* cheese in which 4 ground samples of each were subjected to a force of 1000 times gravity for 40 minutes in a centrifuge. It was found that the *39a* cheese retained 3 per cent more of its moisture than did the *ga* cheese. This difference also points to a firmer binding of water in the former.

The fact that the proteolysis in the *39a* cheese was less thorough than in the *ga* cheese may account for this difference, inasmuch as the greater splitting of the proteins in the latter would free a greater amount of water. However, if the difference in the state of the moisture in the two cheese existed in the earlier stages of ripening it may partly account for the variation in proteolytic cleavage which occurred, since Gortner (21) states that it seems probable that the direction of enzyme action in proteins is largely influenced by the ratio of "bound" to "free" water.

CONCLUSION

It is evident from the data presented that the use of the *ga* starter, a bulgaricus organism with a mycoderm, resulted in a markedly greater development of hydrogen-ion concentration in the cheese during the first day than occurred with the *39a* starter, an old strain of *Lactobacillus bulgaricus*. This great difference in acidity resulted in a more thorough proteolysis and a much harder texture in the *ga* cheese than in the *39a* cheese. It was also indicated that less water was bound by the protein in the *ga* cheese than in the *39a* cheese. The *39a* cheese generally contained a little more moisture than the *ga* cheese, but it is doubtful whether this difference was great enough to be a major factor in the marked deviation in texture which occurred.

A significant point in this consideration is that the results of the preliminary work showed a greater difference in moisture but less difference in texture between the *ga* cheese and the *39a* cheese than existed between the two groups of cheese in table 1. It was also noted that 4 of the 19 pairs of cheese upon which moisture data were obtained showed little or no difference in moisture but a marked difference in texture, while in another pair (428) the *39a* cheese with its superior texture contained 0.6 per cent less moisture than its mate.

It is known that Cheddar cheese becomes soft and mellow as the proteins are changed form insoluble to soluble forms. However, it is at once apparent from these experiments with Swiss cheese, that the production of a desirable soft texture was not necessarily coincident with the highest degree of proteolysis, quite the opposite being the case. In this connection it has been pointed out by Eldredge and Rogers (22) that a difference exists in the texture of the Swiss and Cheddar cheese, although the major chemical changes are similar for both types. The changes in the protein occurring in both cheese have been followed closely by several investigators (23).

It seems probable, therefore, that variations in the colloidal structure of the cheese, as shown by the difference in the binding of the water, exert an important effect upon the texture, but further work will be necessary in order to clear up this phase of the investigation. These experiments indicate, however, that the choice of the starter is an important factor in the control of the hydrogen-ion concentration, ripening, and texture of Swiss cheese.

ACKNOWLEDGMENT

Acknowledgment is due K. J. Matheson and G. P. Sanders who made the cheese in this investigation. Mr. Sanders also determined the moisture content of the cheese and Mr. Matheson made the texture measurements, being the first to prove the differentiation in texture between cheese made with the two starters.

REFERENCES

(1) Orla-Jensen, S.: "Dairy Bacteriology," J. and A. Churchill, London, 1921, p. 128.

(2) Boekhout, F. W. J., and Ott de Vries, J. J.: Rev. Gén. Lait, vii, 385 (1909).

(3) Van Dam, W.: Rev. Gén. Lait, viii, 169 (1910); ix, 151 (1912).

(4) Leitch, R. H.: Proc. World's Dairy Congress, Washington, D. C., 1923, p. 321.

(5) Van Slyke, L. L., and Hart, E. B.: N. Y. (Geneva) Agr. Expt. Sta. Bull. 236 (1903).

(6) Van Slyke, L. L.: N. Y. (Geneva) Agr. Expt. Sta. Bull. 207 (1901).

(7) Watson, P. D.: Ind. Eng. Chem., xix, 1272 (1927).

(8) Bierman, H. R., and Doan, F. J.: Jour. Dairy Sci., vii, 381 (1924).

(9) Golub, W.: Z. anal. Chem., lxx, 119 (1927).

(10) Koch, F. C., and McMeekin, T. L.: Jour. Amer. Chem. Soc., xlvi, 2066 (1924).

(11) Van Slyke, D. D.: Jour. Biol. Chem., lxxi, 235 (1927).

(12) Van Slyke, L. L., and Hart, E. B.: N. Y. (Geneva) Agr. Expt. Sta. Bull. 215, 85 (1902).

(13) Teichert, K.: "Methoden zur Untersuchung von Milch and Milcherzeugnissen," F. Enke, Stuttgart, 1927, p. 417.

(14) Van Slyke, D. D.: Jour. Biol. Chem., xii, 275 (1912).

(15) ORLA-JENSEN, S.: Land. Jahrb. Schweiz, xviii, 319 (1904).
GRATZ, O.: Z. Nahr. Genussm., xxiii, 379 (1912).
BARTHEL, CHR. AND ROSENGREN, L. FR.: Medd. Centralanstalt. försöksvasendet Jordbrucks, No. 219 (1921).

(16) WATSON, P D., AND LEIGHTON, A.: Jour. Dairy Sci., x, 331 (1927).

(17) CLARK, W. M.: In "Fundamentals of Dairy Science" by Associates of Rogers, Chemical Catalog. Co., Inc., 1928, p. 129.

(18) EFFRONT, J.: "Biochemical Catalysts in Life and Industry," John Wiley & Sons, Inc., 1917, p. 626.

(19) VAN SLYKE, L. L., AND BOSWORTH, A. W.: Jour. Biol. Chem., xiv, 231 (1913).

(20) MORAN, T.: Proc. Roy. Soc. (London) 112A, 30 (1926).
THOENES, F.: Biochem. Z., clvii, 174 (1925).

(21) GORTNER, R. A.: "Colloid Symposium Monograph I," University of Wisconsin, 1923, p. 393.

(22) ELDREDGE, E. E., AND ROGERS, L. A.: Centr. Bakt. Parasitenk., II Abt., xl, 5 (1914).

(23) VAN SLYKE, L. L., AND HART, E. B.: N. Y. (Geneva) Agr. Expt. Sta. Bull. 219, (1902): N. Y. (Geneva) Agr. Expt. Sta. Bull. 231, (1903).
WINTERSTEIN, E., AND THORNY, J.: Z. physiol. Chem., xxxvi, 28 (1902); xli, 485 (1904).

THE COMPARATIVE ACCURACY OF THE DIRECT MICROSCOPIC AND AGAR PLATE METHODS IN DETERMINING NUMBERS OF BACTERIA IN MILK*

JAMES D. BREW

Cornell University, Department of Dairy Industry, Ithaca, N. Y.

Laboratory technicians and investigators, interested in the sanitary control of milk supplies, have given considerable thought to the comparative accuracy of the various methods advocated or regularly employed in bacterial control. The cultural plate method has been universally accepted as the standard, primarily because it was the first by which it was possible to make counts of bacteria and because of its official adoption.

The tendency has been, and still is, to regard marked deviations of the results obtained by any new method, from those obtained comparatively by the official agar plate method, as conclusive evidence that the method in question is unsatisfactory. For example, the widespread opinion is that counts by the direct microscopic method are highly variable and are so inaccurate that their application is limited. This conception is well illustrated by the following quotations (1): "The error in estimate is probably quite large." "It should be used for general grouping, rather than for estimates of actual counts." While the first statement is qualified, yet it is easy to understand why the inference is drawn by many readers that counts by the official plate method must be less variable and more accurate. It is well known that, with these thoughts in mind, many Health Officials refuse to attempt any type of laboratory milk control if they are unable to maintain a laboratory equipped for the making of plate counts.

For the most part, conclusions regarding the accuracy of the direct microscopic and agar plate methods have been based upon an analysis, by inspection only, of comparative counts of bac-

---

* Received for publication December 10, 1928.

teria in duplicate samples of milk. Such an analysis may be misleading because the actual differences do not always stand out and because too much reliance is oftentimes placed upon averages.

If it were possible to make a count of the total number of microörganisms in a given quantity of milk, as it is to count the peanuts in a jar, it would be easy to determine the accuracy of any method. But, this being impossible, we are forced to judge the comparative reliability of any two methods by making a long series of estimates of the number of bacteria in duplicate samples. It is then necessary to make a statistical analysis of the data, in order to determine the significance of the variations found.

In the interpretation of any statistical analysis of bacteria counts caution must be exercised in drawing conclusions. There are other factors which should be considered that are not necessarily brought out in the statistical data. For example, it is well known that, in general, a low count milk is more satisfactory than a high count milk; irrespective of the wide variations or of the errors that may be pointed out by those who criticize the methods employed in making counts of bacteria. The greatest criticism, after all, may arise from an erroneous application of the principle of bacterial control due, unquestionably, to a lack of knowledge regarding the true limitations.

In 1917 Brew and Dotterrer (2) reported comparative counts made upon 643 samples of raw market milk as delivered by the producers to the fluid milk plants in Geneva, N. Y. Of these 643 samples counted, 491 represent counts made on plain nutrient agar and 152 on lactose nutrient agar. These studies were made prior to the adoption of the first Standard Methods of Milk Analysis. An effort was made, however, to employ a technique which it was thought would yield the highest plate counts possible.

The media used was made according to the following formula:

Liebigs beef extract	5 grams
Witte's peptone	10 grams
Agar (shreds)	15 grams
Distilled water	1 liter

To this was added 10 grams of lactose in making the lactose nutrient agar. The H-ion concentration, as determined colorimetrically, ranged between pH 6.5 and 7.0.

Three dilutions of 1:100, 1:1000, and 1:10,000 were prepared and triplicate plates were made from each. All plates were incubated at 21°C. for 5 days and then counted with the aid of a magnifying lens, after which they were incubated two additional days at 37°C. and recounted. The count recorded in each case was the higher of the two. In selecting the plates for counting, only those were chosen which had more than 20 and less than 400 colonies (3). There were necessarily a few exceptions to this rule which were noted in the original tables.

Although these comparative studies were made prior to the adoption of the first Standard Methods of Milk Analysis, it is clearly evident that the technique employed was certain to give to the plate method all of the possible advantages commonly recognized up to the year 1927. It is safe to venture the prediction that had the Present Standard Methods been followed explicitly the plate counts would not have compared as favorably as they did (4).

The microscopic counts were made according to the procedure described by Breed and Brew (5). The microscope was adjusted to give a multiplying factor of 300,000 for the computing of the number of bacteria per cubic centimeter. To obtain as great a uniformity as possible the following rules were observed:

1. Where there were few bacteria in number, 100 microscopic fields were counted; when fairly numerous, 30 fields were counted; and if very numerous, 10 or even in some cases only 5 fields were counted. An ocular micrometer was used to aid in counting the most thickly seeded fields.

2. Separate microscopic counts were made of the individual cells per cubic centimeter and of the groups of cells per cubic centimeter. Whenever a group was too large or too dense to make an accurate count of the individuals possible, an estimate was made.

The comparative counts as originally published were arranged in sequence in the order of the count of the individual cells per cubic centimeter. Theoretically the plate count might be expected to fall between the number of groups and the number of cells per cubic centimeter. However, it was found that 27 per cent of the plate counts were actually greater than the corresponding individual counts by the microscopic method and 19 per cent were less than the group count. Therefore 54 per cent of the plate counts fell between the group and the individual cell count. Further than to classify the data into these three groups, no attempt was made at the time to make a statistical analysis in order to ascertain the true mathematical relationships between the two methods as applied simultaneously to miscellaneous samples of market milk.

The writer recently had an opportunity to again review the data and made such an analysis, in coöperation with the Department of Hygiene at the University of California, and reports herein the statistical results. It is unnecessary to repeat the long list of original counts. Any one who is interested in the details is referred to the original data published in Bulletin 439 of the New York Agricultural Experiment Station, Geneva, N. Y. (2).

Comparative mathematical studies were made of the 491 counts obtained on plain agar, and of the 152 counts obtained on lactose agar. It was necessary to eliminate the first 52 counts from the former and the first 11 from the latter, leaving 439 and 141 comparative counts respectively, because of the fact that the number as obtained by the microscopic method on these particular samples was reported as "less than 3000 per cubic centimeter." This simply means that no bacteria were seen in the 100 microscopic fields counted.

STATISTICAL ANALYSIS OF 439 SAMPLES OF MILK

A comparison of the means of the counts by the different methods shows that the plate counts made on plain agar average higher than the group counts (table 1). This may naturally be expected to occur, in general because of the breaking up of colonies in plating. Samples of milk containing organisms which are not adapted to growth in nutrient media, however, may be expected to yield plate counts lower than that indicated by the microscopic group count. This seems to occur most frequently in those cases where the bacteria are few in number.

The count of individual bacteria by the microscopic method averaged 1,278,000 per cubic centimeter. This, as would be naturally expected, is considerably higher than the microscopic group count of 260,000 and plain agar count of 284,000 colonies per cubic centimeter. Since bacteria counts are known to be highly variable it is essential to consider the probable errors[1] of the respective means which are given in table 1.

TABLE 1

*Means and variables of the comparative counts of bacteria in 439 samples of milk (-000 omitted)**

METHOD OF COUNTING	MEAN M	STANDARD DEVIATION σ	COEFFICIENT OF VARIABILITY C
1. Individual microscopic count	1,278 ±207	6,419 ±146	502 ± 82
2. Group microscopic count	260 ± 54	1,667 ± 38	640 ±133
3. Plain agar plate count	284 ± 37	1,150 ± 26	405 ± 54

* The following formulae were used in making the computations:

Mean, $M = \frac{\Sigma X}{N}$, the standard deviation, $\sigma = \sqrt{\frac{\Sigma X^2}{N} - (MX)^2}$.

Coefficient of variability, $C = \frac{\sigma}{M} 100$.

The probable error of the mean $= \pm\, 0.6745 \frac{\sigma}{\sqrt{N}}$; the probable error of the standard deviation $= \pm\, 0.6745 \frac{\sigma}{\sqrt{2N}}$; of the coefficient of variability $= \pm \frac{0.6745}{\sqrt{2N}} \left[1 + 2\left(\frac{C}{100}\right)^2\right]^{\frac{1}{2}}$. M = mean, N = number of cases, ΣX = summation of cases, and $(MX)^2$ = Mean of X^2.

Because of the breaking up of the colonies into smaller components during the process of plating, the count obtained by the agar plate method is usually larger than that of the microscopic

---

[1] The probable error of the difference between two means is based on the formula

$$P.E. \text{ of } D = \sqrt{(P.E._1)^2 + (P.E._2)^2 - 2r_{1,2}\,(P.E._1)\,(P.E._2)}$$

group count. However, the probable errors of both as applied to miscellaneous samples of raw milk are so large that it cannot be stated that there is a significant difference between counts of the "groups" under the microscope and of the colonies on the agar plates. There is a significant difference, as would be expected, however, between these two types of counts and the count of individual bacteria in the same sample of milk.

The standard deviations measure the variability of the three types of counts. The coefficient of variability is an index and makes possible comparisons with the coefficients of variability of other phenomena. Warren (6) has shown that the coefficient of variation for the height of men is 3.8; the value of milk produced per cow, 25.9; feed purchased per cow, 77.7; and profits in farming 456.4.

The coefficient of variation of the counts of groups of bacteria in table 1 by the microscopic method was highest, C. V. = 640.0 ± 133.0. The count of individual bacteria was next highest, C. V. = 502.0 ± 82.0, and the plate count lowest, C. V. = 405.0 ± 54.0. The coefficient of variation of the bacteria counts in miscellaneous raw milk samples under ordinary control, as given in table 1, is about the same or higher than that for profits in farming. Variability in bacteria counts and in farm profits is high as compared to the variability in biological measurements. The fact that bacteria counts are highly variable, however, cannot be justly used as an argument against the accuracy of the methods. Extremely low variability in the field of bacteriology is rare and suggests the difficulties encountered in any numerical studies and further emphasizes the importance of careful statistical analysis.

When the probable errors of the coefficients of variation of the three types of bacteria count are compared it cannot be said that there is a significant difference in the three coefficients of variation. In other words, according to this particular analysis there is no significant difference in the variability of the counts made by either method as applied to miscellaneous samples of raw market milk.

The highest coefficient of correlation exists between the individual (X_1) and group counts (X_2) by the microscopic method, $r_{1,2} = 0.923 \pm 0.004$ (table 2). The lowest correlation is found between the microscopic group (X_2) and plate counts (X_3), $r_{2,3} = 0.678 \pm 0.017$.

The probable errors of the coefficients of correlation are small which indicate that there is a significant difference between each of the three correlations (table 2). The individual and group counts by the microscopic method made on 439 samples of milk are more highly correlated than are the individual microscopic and plate counts, which in turn are more highly correlated than the group microscopic and plate counts. These lower correlations are undoubtedly due to the variability introduced in the microscopic group count by the difficulty in determining what constitutes a separate group and in the agar plate count by the failure of certain organisms to grow or by variations in the extent to which colonies of bacteria break up during plating.

TABLE 2

*Coefficient of correlation between the individual microscopic and group counts and plain agar counts on 439 samples of milk**

Note: 1 = microscopic count of individual cells; 2 = microscopic group count; 3 = agar plate count.

CORRELATION BETWEEN	COEFFICIENT OF CORRELATION
Individual and group counts, $r_{1,2}$	0.923 ±0 004
Individual and plate counts, $r_{1,3}$	0 826 ±0.010
Group and plate counts, $r_{2,3}$	0 678 ±0.017

* The coefficient of correlation, $r_{1,2} = \dfrac{MX_1X_2 - MX_2MX_2}{X_1 \cdot X_1}$.

The probable error of the coefficient of correlation $= \pm 0.6745 \dfrac{(1 - r^2)}{N}$.

STATISTICAL ANALYSIS OF 430 SAMPLES OF MILK

In studying over the original data to ascertain the possible causes for such extremely high coefficients of variation in the 439 samples reported in table 1 it was noticed that nine samples of milk contained very large numbers of bacteria. Some of the plates contained over 1000 colonies and there were so many bacteria in each microscopic field that it was necessary to limit the size and number of fields counted. An ocular micrometer was used and only 10 microscopic fields were counted on each smear of each of these nine samples of milk. Unquestionably such conditions make it impossible to judge either method fairly. Consequently, a mathematical analysis was made with these nine samples eliminated, leaving 430 comparative counts. The results are shown in table 3.

The average number of individual bacteria in the 430 samples by the direct microscopic method was 467,000, as contrasted to 1,278,000 in the 439 samples (table 1). This clearly indicates the important rôle played by a few samples of milk extremely high in bacterial content.

TABLE 3

Means and variabilities of the comparative counts of bacteria in 430 samples of milk (-000 omitted)

METHOD OF COUNTING	MEAN M	STANDARD DEVIATION σ	COEFFICIENT OF VARIABILITY C
1. Individual microscopic count	467 ±38	1,162 ±27	244 ±20
2. Group microscopic count	75 ±10	303 ± 7	402 ±54
3. Plain agar plate count	138 ±11	347 ± 8	251 ±21

The average group count under the microscope was 75,000 in the 430 samples as compared to 260,000 in the 439 samples, while the average plain agar plate count was 138,000 as compared to 284,000. The same relative relationship holds for the three types of counts for the 430 samples (table 3), as for the 439 (table 1). Apparently students should consider the advisability of eliminating a few excessively high counts, when the number of samples analyzed is large. Few conclusions should ever be drawn from a small number of cases of any phenomena as highly variable as bacteria counts.

The coefficient of variation (table 3) of the individual microscopic counts of 430 samples, C. V. = 244.0 ± 20.0 is approximately one-half that for the 439 samples, C. V. = 502.0 ± 82.0

(table 1). Therefore, the elimination of these nine samples reduced the variability about one-half. As previously stated, there was no significant difference in the coefficients of variation of the three types of counts for the 439 samples. But when the nine extremely high counts were disregarded the coefficient of variation of the plain agar plate count is significantly less than that of the microscopic group count.

The higher variability of the group count is doubtless to be explained by the personal equation. What constitutes a group under the microscope is arbitrarily determined. In many samples of milk this presents no serious difficulty. Samples are rather frequently found, however, in which the predominant flora tend to grow in such loosely arranged masses that it is impossible to decide whether or not one is confronted with a large group of organisms or with several smaller groups lying close together.

TABLE 4

*Coefficient of correlation between the individual microscopic and group counts and the plain agar counts on 430 samples of milk**

CORRELATION BETWEEN	COEFFICIENT OF CORRELATION
Individual microscopic and group counts, $r_{1,2}$	0.639 ±0.019
Individual microscopic and plate counts, $r_{1,3}$	0.583 ±0.021
Microscopic group and plate counts, $r_{2,3}$	0.378 ±0.028

* See notes under table 2.

The lower variability in the plate counts, resulting from the elimination of the nine extremely high counts, undoubtedly illustrates the high variability introduced by the crowding of colonies on the agar plates. Where only one plate is prepared the count is apt to be misleading.

Bacteriologists ordinarily consider microscopic counts of individual bacterial cells more variable than plate counts. However, this study indicates that there is no significant difference in variability as applied to miscellaneous samples of raw milk.

The coefficients of correlation between the three types of counts for the 430 samples are consistently less than for the 439 samples (table 4). The correlation, for example, between the individual and group counts $r_{1,2} = 0.639 \pm 0.019$ (table 4) is less than for the 439 samples, $r_{1,2} = 0.923 \pm 0.004$ (table 2).

It is evident that the nine extremely high counts increased the correlation in the 439 samples. Judging from the correlations for the 439 samples bacteriologists might erroneously conclude that a high relationship between the three types of counts would normally be expected (table 2). However, the data for the 430 samples indicate that this relationship may not be so high (table 4). It would not be surprising to find that this relationship, as shown by different series of comparative counts, might be highly variable. For instance, in extremely low count milks it is likely that still poorer correlations will be obtained. The correlation between the individual microscopic and group counts is significantly higher than the correlation between the individual microscopic and plate counts. In other words if a low individual count is obtained by the microscopic method it is quite likely that the microscopic group count would be lower than the agar plate count.

TABLE 5

Means and variables of the comparative counts of bacteria in 141 samples of milk (-000 omitted)

METHOD OF COUNTING	MEAN M	STANDARD DEVIATION σ	COEFFICIENT OF VARIABILITY C
1. Individual microscopic count.	1,506 ±154	2,712 ±109	180 ±20
2. Group microscopic count	243 ± 36	633 ± 25	261 ±40
3. Lactose agar plate count.	512 ± 23	1,283 ± 52	250 ±37

Statistical analysis of counts were made on 141 samples of milk plated on lactose agar to determine the possible effect of the addition of the lactose.

With reference to the magnitude of the comparative means the same general relationship holds. The probable error of each is relatively so small that a significant difference exists between the three types of counts. Referring to the means of table 1 and 3 which contain plain agar plate counts it will be seen that no significant difference can be claimed to exist between the microscopic group and plain agar plate counts. However, a significant difference does exist between the means of these two types of counts in the 141 samples plated on lactose agar. It would appear from this that the addition of lactose tends to enhance the plate counts.

The coefficient of variability (table 5) of the individual microscopic counts of the 141 samples of milk, C. V. = 180.0 ± 20.0, is somewhat less than the variability of either the microscopic group or lactose agar plate counts. The probable errors, however, indicate that a difference does exist but it can scarcely be said to be significant. No significant difference exists in the variability of the microscopic group and lactose agar plate counts, C. V. = 261.0 ± 40.0 and 250.0 ± 37.0 respectively.

TABLE 6

Coefficients of correlation between the individual microscopic and group counts and the lactose agar plate counts on 141 samples of milk

CORRELATION BETWEEN	COEFFICIENT OF CORRELATION
Individual microscopic and group count	0.849 ±0.015
Individual microscopic and plate count	0.823 ±0.018
Microscopic group and plate count	0.754 ±0.024

A larger number of samples should be studied comparatively to better determine the true relationship that exists between counts made by the two methods.

The correlations in table 6 are high and are quite similar to those presented for the 439 counts in table 2. Here again can be seen the influence of a few extremely high counts, there being but three. It was thought unnecessary to ascertain what the effect would be by the elimination of these as was previously done and reported in table 3.

The influence of variability in count upon correlation is better shown if the coefficients of correlations previously discussed (tables 2, 4, and 6) are incorporated in one table (table 7). The first column shows the correlation that exists between the individual microscopic count (X_1) and the microscopic group count (X_2), the individual microscopic count (X_1) and the plain agar plate count (X_3), and between the microscopic group count (X_2) and the plain agar plate count (X_3), as applied to 439 samples of raw market milk. The second column shows the correlations when nine of these 439 counts were eliminated because of the fact that the microscopic fields and the agar plates contained too many organisms to enable just comparisons. The third column shows the correlations that exist when lactose sugar is added to the media.

In comparing the correlations in columns I and II it is clear that the degree of variability plays an important rôle in determining the correlation. Evidently the higher the variability in the numerical bacterial quality of a given raw milk supply the better the correlations obtained between the counts obtained by the direct microscopic and agar plate methods. The elimination of nine counts (column II), representing crowded microscopic fields and agar plates, resulted in a marked decrease in the correlation. It is likely that where the bacterial content of a raw milk supply is uniformly low the correlation between the two methods may even be lower than that indicated in column II. This emphasizes the danger of falling into error in drawing conclusions regarding the existing correlation when only a few samples are involved.

TABLE 7

Coefficients of correlation between the counts of individual bacterial cells and of groups of cells by the microscopic method and of colonies by the agar plate method, showing the influence of high variability and of the addition of lactose to the medium

CORRELATION BETWEEN	439 COUNTS, PLAIN AGAR, I	430 COUNTS, PLAIN AGAR,* II	141 COUNTS, LACTOSE AGAR, III
1. Individual microscopic and group counts	0.923 ±0 004	0 639 ±0 019	0 849 ±0.015
2. Individual microscopic and plate counts	0 826 ±0 010	0 583 ±0 021	0.823 ±0 018
3. Microscopic group and plate counts	0 678 ±0 017	0 378 ±0 028	0 754 ±0 024

* The 430 counts represent the same samples listed in column I except for nine excessively high counts eliminated because of overcrowded microscopic fields and agar plates.

It is evident from these results that the addition of lactose to the medium used in making agar plate counts (column II) would increase the accuracy of this method.

COMPARISON OF THE MEDIAN AND AVERAGES AS MEASURES OF THE CENTRAL TENDENCY OF BACTERIAL COUNTS

The median of a series of variables arranged in sequence is the middle number. It is not influenced by a few excessively high or low values. The arithmetic mean, however, which is the universally used measure of a central tendency may be greatly affected. As each observation is given a weight in proportion to its magnitude, one very high value may more than counterbalance a larger number of the more characteristic observations. These facts are illustrated in table 8.

TABLE 8

Comparison of the median and the mean or average number of bacteria in milk

METHOD OF COUNTING	MEDIAN	AVERAGE
439 samples of milk		
Individual microscopic count	102,000	1,278,000
Group microscopic count	18,000	260,000
Plain agar plate count	39,000	284,000
430 samples of milk		
Individual microscopic count	99,000	467,000
Group microscopic count	15,000	75,000
Plain agar plate count	38,000	138,000
141 samples of milk		
Individual microscopic count	310,000	1,506,000
Group microscopic count	42,000	243,000
Lactose agar plate count	111,000	512,000

The median of the individual microscopic count for the 439 samples is 102,000 while the average is 1,278,000 or 10 times more. The elimination of the nine excessively high counts reduced the median and the average to 99,000 and 467,000 respectively. The median for the 141 samples is 310,000 as contrasted to 1,506,000 the arithmetic mean, a difference of 5 times. The same general relationship holds for both the group and plate counts.

The arithmetic mean is not always a satisfactory measure of the central tendency of bacteria counts. In many cases the median is a better measure. It is possible to have all counts on 49 samples of milk less than 20,000 while one sample may be several million. In judging the true bacterial condition of such a supply it is clear that this one high count may easily lead to erroneous conclusions when based upon arithmetical averages.

SUMMARY

The results of this statistical analysis of counts made by the direct microscopic and agar plate methods show a high degree of variability in both methods, as applied comparatively to rating raw market milk supplies. In other words, there is such a relatively large experimental error in both methods that sanitarians interested in the bacteriological control of milk supplies must avoid the danger of making too fine distinctions in establishing the maximum numerical limits for the various, so-called, grades of milk. Variations of 200 to 300 per cent have been frequently reported in counts made in well controlled laboratories. While it is true that some technicians, under exceptionally well controlled conditions, have been able to obtain smaller percentage variations, it should not be forgotten that we must be governed by limitations arising through practical application where many samples of milk are daily prepared for counting by technicians who do not have time to exercise that extreme care which is possible only in research laboratories where there always exists ample time in which to plate the half dozen or so samples.

The direct microscopic count of individual cells per cubic centimeter varied from 4.8 to 6.2 times greater than the count of the groups of bacteria under the microscope, and from 3 to 4.5 times greater than the agar plate colony count. The plate count on either plain or lactose agar was greater than the corresponding microscopic group counts. This relationship is to be expected in most samples due to the breaking up of the groups into smaller components during the process of plating the milk.

The individual cell count averaged 4.5 times greater than the plain agar plate count but where lactose was added this difference was reduced to 3 times. There was likewise a reduction in the variability as well as a noticeable increase in the correlation. Undoubtedly the addition of lactose to the medium would materially increase the accuracy of the plating method.

Although the microscopic group count was more variable than either the count of individual cells by the microscopic method or the agar plate count, yet the probable error of the coefficients of variability showed little or no significant difference.

There was a better correlation between the counts of the individual bacterial cells and groups of cells per cubic centimeter by the direct microscopic method than between the individual cell count and the agar plate count.

The poorest correlation obtained was that between the microscopic group counts and the agar plate counts. This is undoubtedly largely due to the variations introduced in the former count by the arbitrary manner in which separate groups are determined and by the counting of dead organisms and in the latter by variations in the adaptability of different organisms to growth in cultural media, and also by variations in the breaking up of colonies during plating.

A few excessively high counts may have a marked effect upon the degree of correlation. For example, the elimination from 439 counts in all, of nine which were excessively high and which represented over-crowded microscopic fields and agar plates, resulted in a markedly lower correlation throughout (see table 7). It is conceivable that with raw milk uniformly low in bacterial content that there might be very little if any correlation between the two methods. Although, while showing more or less lack of correlation as applied to individual samples of milk, yet a close agreement may, in general, be expected in the results obtained by the two methods in rating the whole supply.

Arithmetic averages of bacteria counts are apt to be misleading as a measure of the central tendency. One high count among several that were uniformly low would give an erroneous idea of the numerical bacterial quality of a given milk supply. The median, which is the middle number of a series of counts arranged in sequence, is a much fairer measure.

One method gives as reliable a picture of the bacterial condition of any given raw milk supply as does the other. There is no reason for believing that one method is significantly more accurate than the other; nor that one lends itself more advantageously to the dividing of a raw milk supply into classes than the other. The plating method, however, is more applicable to the counting of bacteria in milk where there are only a few thousand present per cubic centimeter.

Irrespective of the wide variations and possible sources of error that may be pointed out, both methods, if properly administered, are sufficiently accurate to insure a marked reduction in the amount of carelessly handled milk, without inflicting an undue hardship upon anyone.

ACKNOWLEDGMENTS

It is desired to acknowledge the valuable suggestions given in the preparation of this manuscript by Dr. Eschscholtzia Lucia of the University of California, Berkeley, California, Dr. H. H. Love and Dr. F. A. Pearson of Cornell University, Ithaca, N. Y.

REFERENCES

(1) Kelly, Ernest and Clement, C. E. Market Milk. John Wiley and Sons, 1923, p. 128.

(2) Brew, J. D., and Dotterrer, W. D. The number of bacteria in milk. New York Agr. Expt. Station Bul. 439. Geneva, N. Y. 1917.

(3) Breed, R. S., and Dotterrer, W. D. The number of colonies allowable on satisfactory agar plates. The New York Agr. Expt. Station Tech. Bul. 53. Geneva, N. Y. 1916.

(4) Robertson, A. H. The relation between bacterial count from milk as obtained by microscopic and plate methods. New York Agr. Expt. Station Tech. Bul. 86. Geneva, N. Y. 1921.

(5) Breed, R. S., and Brew, J. D. The counting of bacteria by the microscope. New York Agr. Expt. Station Tech. Bul. 49. Geneva, N. Y. 1916.

(6) Warren, G. F. Index Numbers of Variability. New York State Col. of Agr., Cornell University. Farm Economics. No. 34. P. 456. May 15, 1926.

THE USE OF DEHYDRATED EGG PRODUCTS IN THE MANUFACTURE OF ICE CREAM*

WILLIAM S. MUELLER[1] AND FORREST C. BUTTON

New Jersey Agricultural Experiment Station

There is little published data on the use of dried egg yolk in ice cream and, to our knowledge, no data have been reported in the literature on the use of dried egg albumin and dried whole egg in ice cream. The greatest value of dried egg yolks in ice cream seems to lie in their ability to reduce the time required to obtain the desired overrun. Depew and Dyer (1), Sommer (2), Dahle and Caulfield (3), and Turnbow and Raffetto (4) agree that dried egg yolk increases the whipping ability of mixes. The use of dried egg yolk in ice cream was investigated by Tracy (5). He added the yolk before and after homogenizing the mix. Since the viscosity was sometimes decreased and other times increased, no definite conclusions as to the effect of the powdered egg yolk upon viscosity of the mix could be drawn. Tracy found that dried egg yolk was detrimental to overrun when added to the mix after homogenization, but favorable results were obtained when the yolk was added to the mix before homogenization. He also found that the addition of the dried yolks produced a smoother texture and greater resistance, but the flavor was objectionable to many. Mojonnier and Troy (6) state that dried egg yolk has a tendency to increase the melting resistance of ice cream. Dahle and Caulfield (3) consider egg yolk as an emulsifying agent rather than a stabilizer. They conclude that dried egg yolk is superior as an emulsifying agent to milk solids-not-fat.

---

* Received for publication December 20, 1928. Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

[1] The data are taken from a thesis presented by William S. Mueller in partial fulfillment of the requirements for the degree of Master of Science, Rutgers University, New Brunswick, New Jersey.

GENERAL PLAN OF EXPERIMENT

The general plan of this investigation was to use each of the dehydrated egg products; namely, yolk, albumin, and whole egg, in an ice cream mix and to determine its relative effect upon each of the following: basic and apparent viscosities of the ice cream mix; overrun; texture; flavor, and melting resistance. The amount of each egg product that was added remained constant throughout the experiment, but the composition of the mixes was varied. The egg products were added to high and low solids mixes.

TABLE 1

Percentage composition of the mixes

MIXES	FAT	MILK SOLIDS—NOT FAT	SUGAR	GELATIN	EGG PRODUCT	TOTAL SOLIDS
High total solids	12	11	15	0 3	1	39 3
Check	12	12	15	0.3		39 3
Low total solids	8	9	14	0.3	1	32.3
Check	8	10	14	0.3		32.3

EXPERIMENTAL METHODS

Preparation of the mixes

One per cent of each egg product was added to high total solids mixes and to low total solids mixes. Forty pound mixes were used. The composition of the mixes is given in table 1. The percentage of milk solids-not-fat was increased in the check mixes in order to have the total solids content equal to that in the egg product mixes. Four batches were always run at one time, and were designated as follows: Mix No. 1, check; mix No. 2, albumin; mix No. 3, yolk; mix No. 4, whole egg. Forty per cent cream, 30 per cent condensed skim milk, and whole milk were the dairy ingredients used. These dairy products were mixed in the pasteurizer.

The best obtainable grades of flaked whole egg, flaked albumin, and yolk manufactured by the spray process were employed. The flaked albumin and whole egg were ground fine in a mill. As the dehydrated yolk was purchased in the powder form no further treatment was necessary for its use. The egg products were added in powder form so as to obtain the greatest possible solubility. The gelatin and egg products were mixed with the sugar and slowly added, at the pasteurizer, to the dairy ingredients when the latter had reached a temperature of 90°F. All mixes were pasteurized at 145°F. for thirty minutes and viscolized at this temperature at 2500 pounds pressure. After viscolization the mixes were immediately cooled to 40°F., and then aged for twenty-four hours in the cold room at 36°F.

Determination of the viscosity

The determination of the apparent and basic viscosities was made with a New Model Stormer Viscosimeter. All viscosity readings were made at 20°C. A 150-gram weight was used and the time required for the cylinder to make 100 revolutions was recorded in tenths of seconds. The formula $V = 4.6\,t - 25$, where t is the time for 100 revolutions, was used in converting seconds to centipoises. Viscosity determinations are reported in centipoises, in order to make all of the results directly comparable and independent of the instrument employed. Viscosity determinations were made on samples before aging, and after aging twenty-four and forty-eight hours at 36°F. The first reading of each unagitated sample was recorded as the apparent viscosity. Four readings were made on each agitated sample and the average of these recorded as basic viscosity. The apparent viscosity was removed from samples for basic viscosity determinations by agitating them for about one-half hour in vacuo, in a side necked filter flask. The agitation was produced by securely fastening the flasks in a small, electrically driven churn.

Method of freezing

After twenty-four hours aging the mixes were frozen in a motor driven horizontal brine freezer. The temperature of the mix was 37°F. at the time it was placed in the freezer. The average brine temperature was −7°F. In order to secure consistent freezing results, the brine was always shut off when the mix had reached the desired consistency in the freezer. The object during freezing was not to draw the cream after it had reached the right consistency for drawing, but after it was certain the maximum overrun had been obtained.

Method of determining the overrun

Overrun determinations were made with a Mojonnier overrun tester. Readings were made every minute during the freezing process, except toward the latter part of the freezing time when overrun readings every half minute were sometimes necessary.

Securing the samples

Four samples were taken from each batch in pint cartons. These were used in determining the quality of the finished product. All samples, with a few exceptions, were drawn at 80 per cent overrun.

Judging the ice cream

The samples were removed from the hardening room from time to time and placed in an electrically refrigerated cabinet. After reaching a good dipping temperature the samples were judged for texture and flavor. No score card was used, but conclusions were drawn from the summarized comments of the judges.

Method of determining melting resistance

The difference in the ability of the samples to withstand the influence of heat was determined by subjecting all samples to the same heat conditions, noting the appearance of the sample and the rate of melting during the melting process. All samples for this determination contained 80 per cent overrun, and were drawn from the freezer into pint paper cartons. Samples were subjected to a room temperature of about 73°F. Pictures were taken immediately after placing the samples upon the wire gauze and at the end of one and one-half and three hours.

EXPERIMENTAL RESULTS

Effect of dehydrated egg products on the apparent and basic viscosity of the ice cream mix

Basic viscosity readings were made to determine whether there was any relation between basic viscosity and whipping ability of the mixes. Apparent viscosity determinations were made mainly for comparison with the basic viscosity.

TABLE 2

Average viscosity for high solids mixes

MIX	UNAGED	AGED 24 HOURS		AGED 48 HOURS	
		Apparent	Basic	Apparent	Basic
	Cp.	*Cp.*	*Cp.*	*Cp.*	*Cp.*
Check	18.35	60.68	28.40	55.30	29.18
Albumin	20.17	90.09	38.29	70.45	37.65
Yolk	19.48	96.07	35.12	79.19	34.43
Whole egg	19.94	81.58	34.16	70.54	33.74

TABLE 3

Percentage increase in viscosity of egg mixes over control mix, for high solids mixes

MIX	INCREASE OF EGG MIX OVER CONTROL MIX IN UNAGED MIX	INCREASE OF EGG MIX OVER CONTROL AFTER 24 HOURS AGING		INCREASE OF EGG MIX OVER CONTROL AFTER 48 HOURS AGING	
		Apparent	Basic	Apparent	Basic
Albumin	10.03	47.97	34.82	26.93	28.71
Yolk	6.27	57.80	23.66	42.68	17.99
Whole egg	8.78	34.00	20.24	27.09	15.62

The average apparent and basic viscosity readings of four series of high total solids mixes are given in centipoises in table 2.

The data in table 2 show that all of the egg products increased the apparent and basic viscosity before aging and after aging twenty-four and forty-eight hours.

Table 3 gives the percentage increase in viscosity of egg mixes over the control mix, in unaged and in twenty-four- and forty-eight- hour aged mixes. The figures show the percentage increase in centipoises. It will be noted that the egg products mixes differ in both apparent and basic viscosity. The viscosity differences and the increase in viscosity of the egg mixes over the control mix, were probably due to concentration differences. As would be expected there is very little difference in the viscosities of the unaged mixes. Table 3 also shows that the egg products give the greatest increase in viscosity after twenty-four hours aging. At that time the whole egg mix had the lowest apparent and basic viscosity and the yolk mix had the greatest apparent viscosity, while the albumin mix had the greatest basic viscosity of all the egg mixes. There was no marked change in basic viscosity after forty-eight hours aging over the mixes which were aged twenty-four hours. This agrees with the observations of Leighton and Willams (7) that the basic viscosity of a mix shows no marked change after twenty-four hours aging. An increase in apparent viscosity after forty-eight hours aging is usually expected. How-

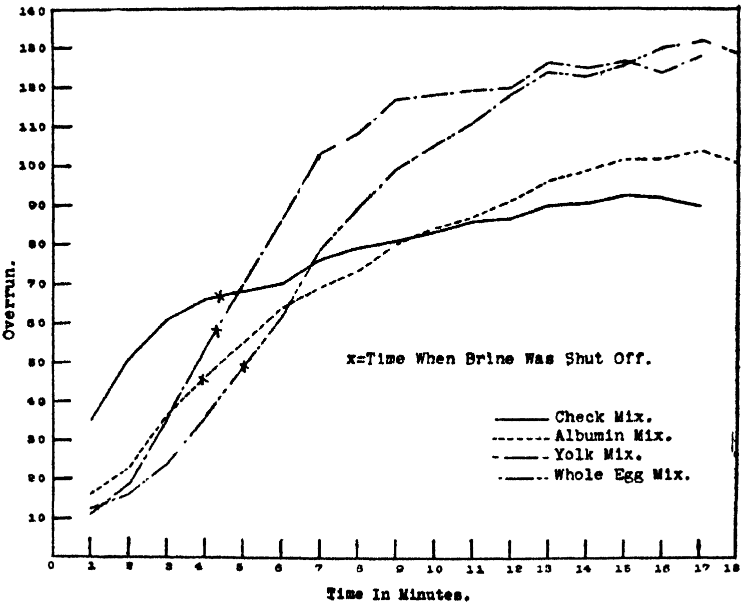


FIG. 1. SHOWING EFFECT ON OVERRUN OF 1 PER CENT DEHYDRATED EGG PRODUCT IN A MIX CONTAINING 39.3 PER CENT TOTAL SOLIDS

ever this was not the case in this experiment, which in all probability was due to the small amount of gelatin used.

The viscosity readings for the low solids series were lower than those for the high solids series, because of the low solids content of the former.

Effect of dehydrated egg products on overrun

The average overrun readings of two series of high total solids mixes are plotted in figure 1 for every minute during the freezing process. Percentage overrun is represented as the ordinate and freezing time in minutes as the abscissa. Figure 1 shows that the check and albumin curves follow each other closely, as do the yolk and whole egg curves. It will be noted that air was incorporated more readily in the yolk and whole egg mixes than in the albumin and check mixes.

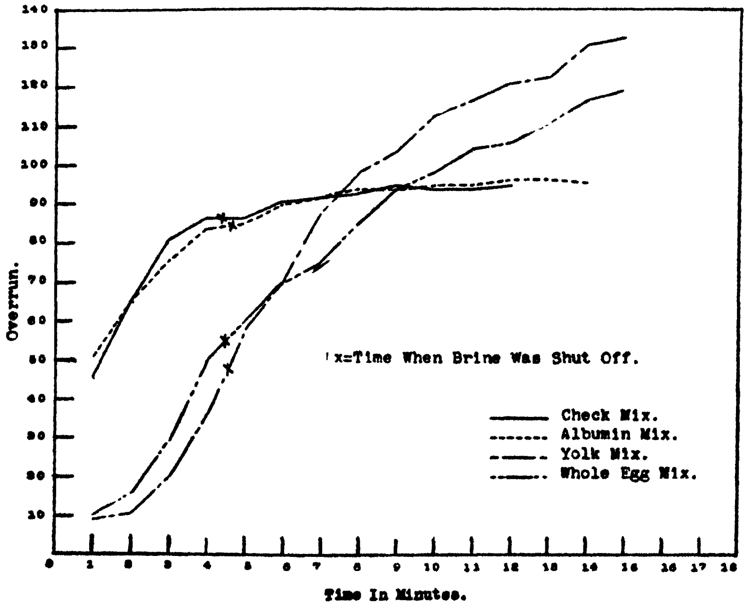


FIG. 2. SHOWING EFFECT ON OVERRUN OF 1 PER CENT DEHYDRATED EGG PRODUCT IN A MIX CONTAINING 32.3 PER CENT TOTAL SOLIDS

The effect of each of the egg products on overrun when added to a low total solids mix is shown in figure 2. Overrun determinations are the average of two series. The overrun curves of figure 2, are very similar to those on figure 1 with the exception of the check and albumin mixes which attained their maximum overrun sooner in the low total solids mixes than in the high total solids mixes. For comparison, the time required for each mix to attain 100 per cent overrun, to attain its maximum overrun, and the maximum overrun attained, are given for both low and high solids mixes in table 4.

As a large number of ice cream manufactures draw the ice cream at 100 per cent overrun, it is interesting to note, in table 4, the time required for each mix to reach that overrun. The check mix did not reach 100 per cent overrun in the high total solids series, and the check and the albumin mixes did not reach 100 per cent overrun in the low solids series. Less time was required to reach 100 per cent overrun when yolk and whole egg were added to high solids mixes, than when added to low solid mixes. There was no marked difference between the low and high solids series in the time required to reach maximum overrun and in the maximum overrun attained.

TABLE 4

Amount of overrun and time required to obtain it

MIX	MAXIMUM OVERRUN		TIME REQUIRED TO REACH MAXIMUM OVERRUN		TIME REQUIRED TO REACH 100 PER CENT OVERRUN	
	Low solids	High solids	Low solids	High solids	Low solids	High solids
	per cent	*per cent*	*minutes*	*minutes*	*minutes*	*minutes*
Check	95	93	9	15		
Albumin	97	102	12	15		14
Yolk	133	127	15	15	8.3	7
Whole egg	119	130	15	16	10.5	9

The overrun data on egg yolk, obtained in this experiment, corroborates the results of other investigators (1, 2, 3, 4) studying the effect of egg yolk on whipping ability in that dried egg yolk facilitates the incorporation of air. The fact that the albumin did not increase whipping ability whereas whole egg did, indicates that the increase in whipping ability was due to the yolk. How the dried yolk increases whipping ability is not definitely known. The effect of egg yolks upon the whipping ability of mixes, may be partly due to the effect the yolk has on the surface tension of the mix. Dahlberg and Hening (8) say that theoretically a low surface tension should be related to ease of whipping, but no experimental evidence has been reported to establish this relationship for cream or ice cream mixes. Turnbow (4) believes that the lower the surface tension, the faster the mix whips in the freezer. Since practically all emulsoid colloids lower the surface tension, it is not unlikely that the dried yolk has a similar effect, thus increasing the whipping ability of ice cream mixes.

The ability of the egg yolk to make the mix whip faster in the freezer is also probably partly due to its emulsifying power. Bancroft (9) mentions that egg yolks are a good emulsifying agent.

What constitutents of egg yolk are responsible for the rapid incorporation of air is not definitely known. Turnbow (4) states that the salts present in eggs may have a bearing upon the whipping ability of the mix.

According to the viscosity and overrun data secured in this experiment, no relationship existed between either apparent or basic viscosity and whipping ability of the mixes.

Effect of dehydrated egg products upon the melting resistance and retention of air of the ice cream

As the melting resistance of ice cream is of great economic importance to the ice cream industry, the melting resistance of the ice cream containing the egg products when added to both low and high solid mixes was determined. Plates 1 and 2 inclusive show the results of this experiment. Plate 1, Fig. 1 shows the high total solids ice cream at the beginning of the experiment, and figures 2 and 3 show the ice cream after being subjected for 1½ and 3 hours, respectively to a room temperature of 73°F. Plate 2, Fig. 1 shows the low total solids ice cream at the beginning of the experiment, and Figures 2 and 3 show the ice cream after being subjected for 1½ and 3 hours, respectively, to a room temperature of 73°F.

The pictures show rather conclusively that the use of dehydrated egg products in ice cream does not affect the melting resistance to any noticeable extent. In making the melting resistance determinations, however, it was noted that the filtrate from the check and albumin samples contained very little air, while the filtrate from the yolk and whole egg samples contained considerable foam. Thus, the height of the filtrate within the cylinders does not indicate the rate of melting. This difference in retention of air was more pronounced in the low total solids mixes than in the high total solids mixes. The relative size of the air cells and the thickness of their walls in check, albumin, yolk, and whole egg samples are shown by the following data:

	AIR CELL	WALL
	inches	*inches*
Albumin	2 00	0.24
Check	0.82	0 20
Whole egg	0.24	0.14
Yolk	0.16	0.10

The microscopic examinations showed that as the size of the air cell decreases, the width of the film surrounding the air cell increases. According to Bancroft (9) in order to get a fairly permanent foam the surface film must either be sufficiently viscous in itself or must be stabilized in some way. He says that this can be done by introducing a solid powder into the interface. It is not unlikely that the egg yolk powder stabilizes the surface film in this way, thus accounting for the ability of the yolk and whole egg ice cream to retain air while melting. The high overrun obtained in the yolk and whole egg mixes may also be partly due to the solid particles of yolk stabilizing the surface film surrounding the air cells.

Effect of dehydrated egg products upon flavor and texture of the ice cream

The relative placing of the high and low solids series is given in table 5.

The placings given are the average for eight scorings. It was evident that yolk and whole egg improved the texture while albumin was injurious to texture. This improvement in texture attributed to the use of egg yolk, was not due to any additional solids because all mixes had the same total solids content. The improvement in texture was more noticeable in the low solids mixes.

The albumin samples left a slightly disagreeable albumin aftertaste, whereas the whole egg samples had a characteristic egg flavor. The egg yolk did not seem to be detrimental to flavor, as it was preferred over the check sample in the high solid series. Since the egg flavor was more pronounced in the low solid series than in the high solid series, the check sample had preference over the yolk sample in the low solid series.

TABLE 5

Placings of high and low solids samples

SAMPLE	RELATIVE RATING AS TO FLAVOR		RELATIVE RATING AS TO TEXTURE	
	High solids	Low solids	High solids	Low solids
Check	2nd	1st	3rd	3rd
Albumin	4th	4th	4th	4th
Yolk	1st	2nd	1st	1st
Whole egg	3rd	3rd	2nd	2nd

Because of individual tastes, it is difficult to say what percentage of egg products may be used in ice cream without injuring the palatability. Greatest preference was shown in the yolk samples and least for the albumin samples. The preferences for the whole egg and check samples were about equally divided. The palatability of the yolk sample was due to its smooth, creamy consistency, rich color, and characteristic flavor. These attributes which were contributive to the palatability of the yolk samples were less pronounced in the whole egg samples. The albumin samples did not have the smooth, creamy consistency, and a slight albumin aftertaste was noticed.

SUMMARY

1. One per cent dried egg yolk increased the whipping properties of the mix and thereby shortened the freezing time per batch.
2. Dried egg yolk also improved the texture of the ice cream.
3. The dried egg yolk did not contribute a stabilizing effect to the ice cream.
4. One per cent dried whole egg was only slightly less effective than the egg yolk in increasing the whipping qualities, in shortening freezing time and in improving texture of the ice cream. Like egg yolk, it did not contribute a stabilizing effect to the ice cream.
5. One per cent dried egg yolk did not injure the flavor of the ice cream, whereas 1 per cent dried whole egg was slightly injurious to the flavor of the ice cream.
6. One per cent dried egg albumin was detrimental to the mix. It possessed no stabilizing properties and was injurious to the texture and the flavor of the ice cream.
7. In the yolk and whole egg ice cream, the improvement in texture was more noticeable in the low total solids ice cream.
8. Less time was required to reach 100 per cent overrun when the dried yolk and whole egg were added to high total solids mixes, than when added to low total solids mixes.
9. The flavors imparted in the ice cream by the use of the egg products, were more pronounced in the low total solids ice cream than in the high total solids ice cream.
10. The dehydrated egg products increased the apparent viscosity while the basic viscosity was only slightly increased.
11. In this experiment there was no relationship between apparent and basic viscosities of the mix and the ability to obtain overrun.

REFERENCES

(1) DePew, H. F., and Dyer, S. W. 1925 The manufacture of ice creams. The Creamery and Milk Plant Monthly, xiv, no. 8, 87.

(2) Sommer, H. H. 1927 Factors that affect the whipping quality of ice cream mixes. Ice Cream Review, xi, no. 2, 86.

(3) Dahle, C. D., and Caulfield, W. J. 1928 Time, temperature, overrun of mixes. Ice Cream Field, xii, no. 6, 11.
(4) Turnbow, G. D., and Raffetto, L. A. 1928 Ice Cream. John Wiley & Sons, Inc., New York, 112, 115, 121, 133 and 189.
(5) Tracy, P. H. 1924 The non-milk products in relation to quality of ice cream. The Creamery and Milk Plant Monthly, xiii, no. 2, 77.
(6) Mojonnier, T., and Troy H. C. 1925 The Technical Control of Dairy Products, 2nd Edition, Mojonnier Bros., Chicago, Ill., 297.
(7) Leighton, A., and Williams, O. E. 1927 The basic viscosity of ice cream mixes. Jour. Phys. Chem., xxxi, 596–600.
(8) Dahlberg, A. C., and Hening, J. C. 1925 Viscosity, surface tension and whipping properties of milk and cream. New York State Agri. Exp. Sta., Technical Bul. 113, 38.
(9) Bancroft, W. D. 1921 Applied Colloid Chemistry. McGraw-Hill Book Co., Inc., New York, 260–86, 270

PLATE 1

SHOWING EFFECT OF DEHYDRATED EGG PRODUCTS IN ICE CREAM ON MELTING RESISTANCE

Samples from left to right are check, albumin, yolk and whole egg

Figures 1, 2, 3 showing ice cream of high solids content after exposure to temperature of 73°F. for none, one and one-half and three hours.

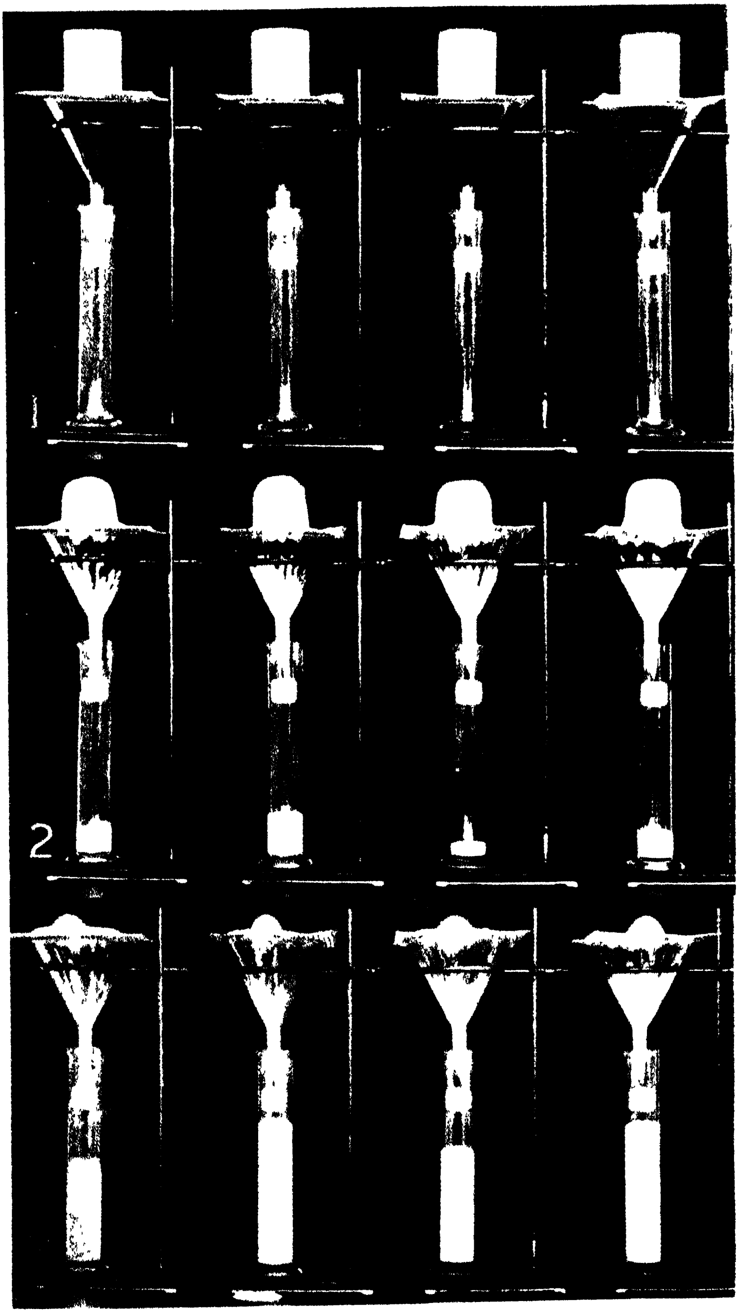
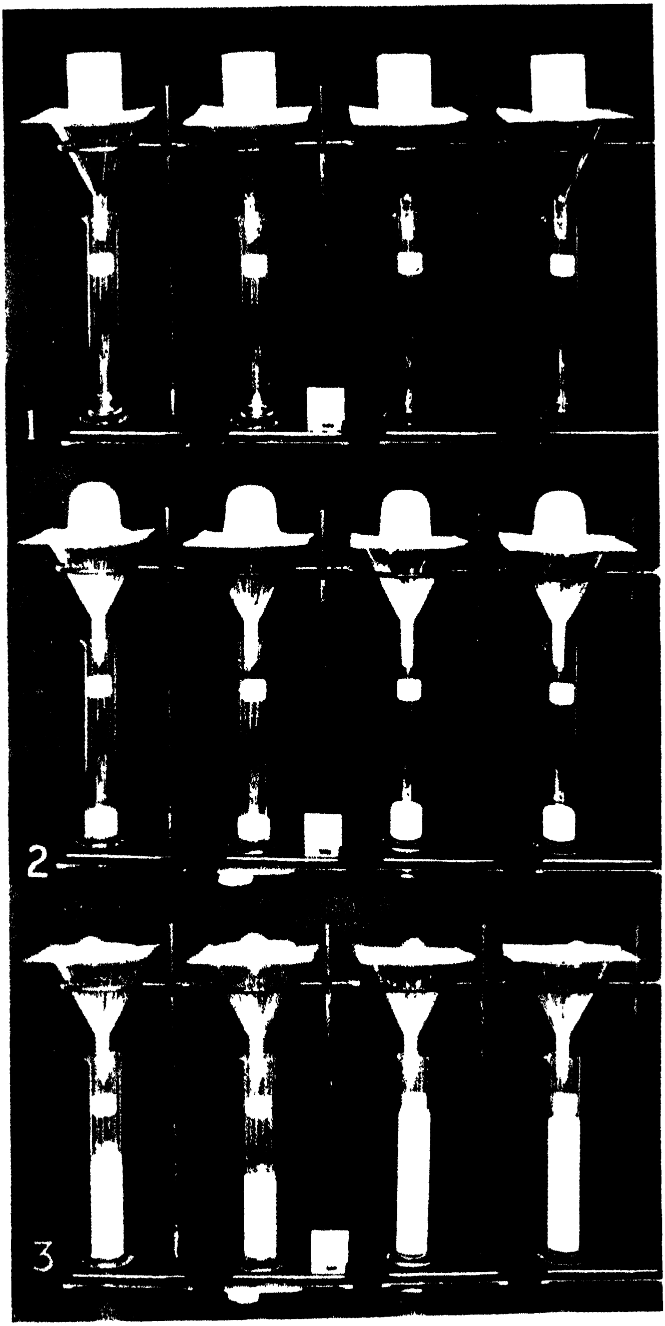

2

PLATE 2

SHOWING EFFECT OF DEHYDRATED EGG PRODUCTS IN ICE CREAM ON MELTING RESISTANCE

Samples from left to right are check, albumin, yolk and whole egg

Figures 1, 2, 3 showing ice cream of low solids content after exposure to temperature of 73°F. for none, one and one-half and three hours


1
2
3

A SUGGESTED SIMPLIFICATION OF THE PRESENT SYSTEM OF OFFICIAL TESTING WITH THE DAIRY BREEDS OF CATTLE*

R. B. BECKER AND P. C. McGILLIARD

Department of Dairy Husbandry, Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma

Nearly a half-century ago, Solomon Hoxie (1) devised a new scheme for the improvement of breeds of dairy cattle. Prior to 1880, improvement of the dairy breeds was brought about in Europe and the adjacent islands through natural selection, by enforcement of laws (2) that prevented further introduction of foreign cattle, by application of standards of perfection (the score card) in the show ring, and by development of the pedigree registers (3) which enabled those persons in the cattle business to guard against the use of breeding animals from inferior ancestors. Hoxie observed, during journeys abroad and through experience with his own cattle, that even within one breed, cows varied widely in their producing ability. He conceived the idea (4) that breed associations have a dual responsibility, namely: (*a*) to guard the purity of blood lines through maintenance of a pedigree register, and also (*b*) to promote the better classes of purebred dairy cows by recording their merits (milk production and conformation) in an advanced or "Main Register." The purpose of the Advanced Register, as he stated (5), was ". . . . to increase and maintain public interest in our breed . . . .; to inaugurate a general work of improvement in the breed . . . .; to collect observations upon which a science of cattle culture . . . . may be built." A registered cow of this breed might gain entrance to the Main Register by completing a stipulated milk record and then by passing inspection under a duly appointed representative of the breed association.

The American Jersey Cattle Club (6) took over the publication of private churn records made by cows of this breed, while

---

* Received for publication December 24, 1928.

the Guernsey (2) and Ayrshire associations fostered "Home Dairy Tests," and competitions. The production records under all of these systems were virtually private records of which the public was somewhat skeptical. However, a turning point in the method of obtaining the records was near at hand.

Dr. S. M. Babcock (7) invented a method of determining the butterfat content of milk which was both accurate and easily operated, which he gave to the public in 1890. On March 14, 1893, the Holstein-Friesian Association (8) sanctioned the use of the Babcock test, and in 1894 called upon the state agricultural colleges and experiment stations to supervise the production records. The American Guernsey Cattle Club (2) established an Advanced Registry in 1901 based upon yearly butterfat production, the official tests likewise being supervised by the experiment stations. These steps marked the beginning of the present method of official testing of cows for the Advanced Registry.

The experiment stations soon felt the necessity of a uniform set of rules to govern the work of the test supervisors in securing the official butterfat tests of cows each month. To meet this need, a committee representing the present American Dairy Science Association formulated a set of rules (9) in 1914, to govern the work of the test supervisors employed by the experiment stations. By observing these rules, the methods of supervising official tests are uniform in all the states.

Except for minor changes, the Advanced Registry system remains virtually unchanged up to the present day.

The idea of using production records in the dairy cattle industry has not been confined to the United States. In 1895, a cow testing society was organized by a group of farmers at Vejen, in the province of Jutland, Denmark (10), the purpose of which was to determine the milk and butterfat yields and feed consumption of all cows owned by the members. This type of record association in which the dairymen joined together to employ a tester who secured the records of all cows in their herds, became known as a Cow Testing Association (Dairy Herd Improvement Association). This type of recording society (10) was organized in

Newaygo County, Michigan in 1905. The cow testing association movement was not confined to purebred cows only, but included all classes and breeds of cows. The movement spread rapidly to nearly every state in the union, and is now an important agent in the dairy industry.

The first Milk Recording Society in Scotland (11) was formed in 1903. As time passed other societies were formed, which later federated into the Scottish Milk Records Association. Changes were made gradually in the conduct of the work. Since 1914, cows are divided into classes upon the basis of production records. The records of the best cows admit them to Class I, and are published in the annual report of the association. The medium producing cows (Class II) are considered fair commercial animals, but their records of production are not eligible for publication. The lower producers, or Class III cows, are looked upon with discredit; the number and percentage of these in each herd are published annually by the association. The conduct of surprise check tests and direction of the milk recorders (test supervisors) are under the control of the central office of the Scottish Milk Records Association.

A careful study of the method employed in the Scottish system of securing production records, discloses the fact that it in reality combines the functions of the Advanced Registry and Cow Testing Association. Every cow in the herd is tested. Supervision of the records is strict, and is impartially superintended from the central office of the Scottish association. Records of high cows (Class I) are published, since the desirable breeding animals usually are found among them. Emphasis is placed upon locating the low producers (Class III) and giving such publicity to their presence in herds that the owners may be induced to discard them. The middle class cows (Class II) are recognized as having some value as milk producers. The fact that their records are not published induces the public to regard them as less valuable breeding animals.

The Herd Test in the United States is of recent origin (12), although it was discussed as early as 1920. Archibald Kirkpatrick of Scotland, criticized our system of testing, as shown in editorial comment in "The Ayrshire Digest," July 15, 1923. Since officers of the Ayrshire Breeders Association planned to present a definite herd test system at the 1924 annual meeting, they invited Professor O. E. Reed to discuss the Scottish Milk Record Association system, as he observed it. A Herd Test Committee was appointed September 17, 1924. The first Herd Test rules appeared in "The Ayrshire Digest," May, 1925. The new system was officially adopted on June 10, 1925, and though used for three years, is still in the experimental stage. All cows in the herd are tested and the records of the higher producing cows are published. The method of supervising the records is less strict than either that in Scotland, or in official testing in the United States. No inducement is offered the owner by the Association to dispose of the low producing cows.

The Holstein-Friesian Association (13) added an entirely new and far-reaching feature to the Herd Improvement Register, adopted by them, embodied in the following rule:

> *Omissions from Test.*—6 . . . . . Also, the record of any cow in the herd may be omitted from the Herd Certificate, and from the Herd Improvement Register, provided that, before the close of the eleventh month of the herd test year, her Certificate of Registry has been surrendered to the Superintendent of Advanced Registry for transmission to the Secretary of the Holstein-Friesian Association of America for cancellation.

The American Jersey Cattle Club (14) instituted a Herd Improvement Register, while the American Guernsey Cattle Club passed the matter over for consideration another year.

PURPOSES OF THE ADVANCED REGISTRY

The purposes of the Advanced Registry have been given, as outlined by the founder—Solomon Hoxie. Briefly, the intent is to locate the high producing cows and the blood lines that transmit high production, and to popularize them in anticipation that breeding animals selected from their midst may bring improvement to the breeds.

PURPOSES OF THE COW TESTING ASSOCIATION (D. H. I. A.)

The purpose of the cow testing association, so far as the breed associations are concerned, is to locate the good cows that merit being placed on Advanced Registry test in later lactations. It also aids the dairy farmer to locate the unprofitable cows in his herd, whether purebred, grade, or common cows, that they may be culled, reducing the numbers of such cows in the breeding herd. Full use of the C. T. A. records enables owners to feed to better advantage, and aids in the business management of dairy herds.

PURPOSES OF THE HERD IMPROVEMENT REGISTER

As yet organized, the Herd Improvement Register has contributed nothing new to our system of testing, except in the one instance already noted. The records are not as full nor as useful to the breeder as are those of the C. T. A. (Dairy Herd Improvement Association), nor do they have the prestige of the Advanced Registry records because of less strict supervision of the records. It is recognized that the new system is in the experimental stage, and that from it may evolve a new and better system that will render the desired service to the dairy cattle industry in the future. In fact, the purpose of this paper is to give an historical background and criticism, upon which to build such a proposed improved system of official testing. The proposed system is not new. It is supported in every detail by experimental evidence, and due credit is given to the original references for those who care to study the problem.

THE PROPOSED PLAN WHICH COMBINES THE ADVANCED REGISTRY AND HERD IMPROVEMENT REGISTER INTO A SINGLE SYSTEM OF SUPERVISION

Cows shall be divided into three classes on the basis of their records of production during the Herd Test year, as follows:

Class I cows and heifers shall include all those that have completed a production record of 290.5 pounds of butterfat in 365 consecutive days as two year olds, or of 400 pounds of butterfat at five years of age (the rate of increase being 0.1 pound of butterfat per day older than two years of age at date of last calving).

Class II cows and heifers shall have produced less than the requirements to qualify for Class I, but more than sufficient to fall in Class III.

Class III cows and heifers shall include all those that have produced less than 195.2 pounds of butterfat as two year olds, or 250 pounds of butterfat at mature age of five years or over (the rate of increase being 0.05 pound of butterfat per day older than two years of age at time of last calving).

Six bi-monthly one-day, and one surprise check tests shall constitute the regular number of tests for Herd Improvement Register purposes. Automatic retests shall be made at the expense of the owner. The Superintendent of Advanced Registry of the breed association, may direct additional check tests, which shall be made at the expense of the breed association.

Any cow or cows may be placed on Advanced Registry test at the option of the owner, by applying for the necessary additional number of tests, the entire supervision to be fully in compliance with the uniform rules (15) for official testing. The Advanced Registry period of test shall include only one lactation, whereas the period for the Herd Test shall be a fiscal year.

The owner or his agent shall keep daily milk weights of all cows on Advanced Registry and Herd Improvement Register tests, submitting monthly milk reports to the breed association of all cows on Advanced Registry test. Milk records of cows on Herd Improvement Register test shall be submitted to the Superintendent of Advanced Registry, if he desires.

All tests shall be carried out in accordance with the uniform rules of the American Dairy Science Association (15), including the use of the preliminary dry milking, except that in the Herd Test alone, the maximum number of milkings supervised in one day may be not over 40, duplicate samples need not be tested, and that two cows may be milked simultaneously provided that both are in full sight of the supervisor at all times.

The Advanced Registry and Herd Improvement Register tests shall in no way overlap on the cow testing association (Dairy

Herd Improvement Association) except in so far as the tester in his spare time may be employed by the state superintendent of official testing in the conduct of official tests. In such case, he shall not conduct tests in the herd of any member of the association which employs him, nor upon cows in which a member has a financial interest, except with the full knowledge of the state superintendent of official testing concerning these facts. He shall receive pay only through the office of the state superintendent of official testing.

All registered cows and heifers in the herd that have borne one or more calves, or that have aborted, shall be tested in the Herd Improvement Register. Animals past twelve years of age that have qualified for the Advanced Registry in the 305 or 365 day divisions, shall be tested, but their records may be omitted from computation of the average production of the herd, at the option of the owner. Cows or heifers not making a satisfactory production, may be omitted from the computed herd average only in case the owner sends their registration certificates to the Superintendent of Advanced Registry prior to the completion of the eleventh month of the herd-test year, for transmission to the Secretary of the breed association for cancellation. Production records shall be used on all registered cows and heifers added to, or removed from the herd during the course of the herd-test year.

The Herd Improvement Register certificate issued by the breed association at the completion of the year, shall bear a statement of the following facts, namely

a. Name and address of herd owner.

b. The "average production" in pounds of milk, butterfat, percentage of fat, and average length of lactation of the entire herd (with such exceptions as previously noted) as computed by the "lactation average," "Danish cow-feed-days" method, the "cow month" method (16), or such other uniform method as may be officially adopted by the American Dairy Science Association for this purpose.

c. Total number of cows and heifers in milk in the herd during the year (to include actual number of complete and partial lactations).

d. Names and records of all Class I cows and heifers.

e. Names of all Class II cows and heifers.

f. Names of all Class III cows and heifers retained in the herd as registered animals. This is to include all those whose registration papers were not transmitted to the breed association for cancellation as previously noted.

A SUGGESTED SYSTEM OF SELECTIVE REGISTRATION BASED ON THE HERD IMPROVEMENT REGISTER

In addition to, and entirely separate from the above plan, it is suggested that a system of selective registration be installed, based upon the above system of production records. The steps in such a system are briefly outlined below.

Male and female progeny from Class I registered cows and heifers, sired by a registered bull of that breed, are eligible to registry under the rules (17) of the breed association involved.

Female progeny from Class II registered cows and heifers are eligible for registry in accordance with the above provisions. Male progeny from Class II registered cows and heifers may be eligible to registration only if sired by a registered bull whose dam has a Herd Test or Advanced Registry record exceeding by 25 per cent the minimum butterfat requirement for her age.

Female progeny from Class III registered cows and heifers may be eligible to registry, only if sired by a bull whose dam has a Herd Test or Advanced Registry record exceeding by 25 per cent the butterfat requirement for her age. No other progeny of Class III registered cows and heifers are eligible to registry, but birth certificates showing color sketch, nose print, ear tattoo or other permanent identifying marks may be placed on file in the breed association office, permitting the dam a period of 24 months from date of birth of the calf in which to satisfactorily increase her record to qualify in Class II or Class I.

Birth certificates may be filed on all unregistered progeny of registered parents, the registration papers not being issued until such time as the owner of the dam may have qualified her in the Herd Improvement Register. In no case shall a period in excess of forty-eight months be allowed for this purpose.

DISCUSSION IN SUPPORT OF THE PROPOSED PLAN, AND OF THE SYSTEM OF SELECTIVE REGISTRATION BASED THEREON

The accuracy of bi-monthly and one-day tests, when accompanied by accurate calving, breeding and dry dates, has been demonstrated as sufficiently accurate for Herd Improvement Register purposes, by the work of McDowell (18), Copeland (19), Petersen (20), and unpublished studies made by H. W. Norton, Jr. Where no preliminary dry milking was taken, McDowell found an average variation of 3.8 per cent from the known actual production of 70 cows in the Minnesota Experiment Station herd, while Copeland's study of 500 Register of Merit records secured under official testing conditions showed a variation of only 1.21 per cent in records re-computed by means of the bi-monthly tests. The use of the preliminary dry milking is considered a safeguard in securing official records under the monthly test plan (21, 22, 23, 24), and is even more important in safeguarding records under the bi-monthly plan of supervision with one-day tests. Sentiment was expressed in favor of the preliminary dry milking with any production records which receive the sanction of the breed association at a recent breeders' meeting (25). The preliminary dry milking is required by the administration in some states (26) before the state superintendent of official testing is permitted to attach his signature to the reports of tests. The sentiment in European countries, where the preliminary dry milking is not employed, favors frequent tests (at 14 to 28 day intervals) safeguarded by a number of (two or more) surprise check tests during the year.

Evidence in support of the suggested system of selective registration, based upon the idea of Class I, Class II, and Class III registered cows and heifers, and eligibility of their offspring to registration, is somewhat more scattered, but is equally specific.

In Scotland (11, 27) marked reduction in numbers of Class III cows and heifers, and a marked increase in those qualifying for Class I, under the system of the Scottish Milk Records Association, is shown in table 1.

Studies made by McDowell and Parker (28) have shown a definite correlation between the production of dams and their daughters, and that there is a greater tendency for the offspring of purebred dams to be high producers than for the offspring of grade dams. An historical review of the Purdue University dairy herd by Fairchild (29) and of the Kentucky Jersey herd by Hooper (30) have shown both foundation cows and bulls to have an influence on the quality of the offspring.

The fact that the factors governing milk production and butterfat percentage are inherited independently, has been generally accepted. Many studies have been made relative to the transmission of butterfat percentage from parent to offspring. Copeland (31) has shown that although the characteristic average fat percentage in milk of the Jersey breed has changed but little, the parents have a marked influence on the fat percentage in the milk of their offspring. He concluded that "improvement in total butterfat production can be accomplished by selecting for high butterfat percentage as well as selection for large milk yields." Burrington and White (32) studied two Holstein female families in the Connecticut station dairy herd, and found that although the same bulls had been used on both families, the characteristic fat percentage in the two families had remained apart through five generations of females.

TABLE 1

Improvement among dairy cows in Scotland, under the operation of the Scottish Milk Records Association system of records

YEAR	NUMBER OF COWS	PERCENTAGE OF COWS QUALIFYING	
		In Class I	In Class III
1914	26,424	39.5	9
1918	17,827	49	5 5
1922	27,275	63	2
1924	27,957	65	3
1927	29,459	65.7	2 2

Investigations by the Danish government showed that with cows of equal butterfat production, those having the higher percentage of fat in the milk, produced butterfat more economically.

As the result of this study, the Danish government determined to raise the butterfat percentage typical of the Red Danish breed of dairy cattle by paying federal grants in coöperative bull associations only for bulls whose dams have records showing a certain percentage of butterfat in the milk. Faber (33) cited the influence of such a policy, upon the cattle of Funen (Denmark), as shown in tables 2 and 3.

TABLE 2

Average yields of dams of bulls bought by coöperative societies

YEAR	DAMS			DAMS' DAMS			SIRES' DAMS		
	Milk	Test	Butter	Milk	Test	Butter	Milk	Test	Butter
1905–1906	9,244	3.59	367	9,137	3.53	365	9,038	3.67	372
1911–1912	9,339	3.95	414	9,271	3.76	387	9,381	3.75	389
1917–1918	8,802	4.11	407	9,172	3 92	400	9,350	4.13	431

TABLE 3

Average records of all cows on Funen belonging to members of milk recording societies

YEAR	NUMBER OF COWS	MILK	FAT PERCENTAGE	"BUTTER"
1899–1900	5,467	6,822	3.36	225
1902–1903	17,662	7,410	3 41	282
1911–1912	30,757	7,667	3.52	301
1915–1916	40,116	7,938	3.55	323

Faber stated further that

> The records of milk recording societies proved that the character of high milk yield was hereditary and could be transmitted from a cow to her progeny. . . . . When a large milk yield is desired and the richness is not of so great importance, the object . . . . is more easily attained. Mr. Morkeberg believes that "the capacity to yield much milk and the capacity to yield rich milk are two different characters, both hereditary but inherited the one independent of the other." If that is so, it should be easier to develop just one than both.

Many other references could be cited in support of the inheritance of the characters for high milk and butterfat production. One further reference in support of selective registration on the basis of production records, may be taken from the Island of Guernsey (34). For a number of years, no bull on the Island of Guernsey could qualify for public service unless his dam or granddam had an Advanced Registry record of 360 pounds of butterfat in 365 days. This standard has been increased at intervals and is now 450 pounds of butterfat, with possibility of being raised still higher. Savage (34) stated that "The effect of the rigid selection in force did not become apparent until about 1920, but since that time there has been a gradual progress in both milk and butterfat results (in table 4)."

The Herd Improvement Register is still in the experimental stage in the United States. A wonderful future has been prophesied (35, 36) for it. In the mean time, it is in the interest of this new form of herd test that it be so conducted as to merit public confidence and accomplish the purposes for which it is intended. A definite incentive must be offered to induce breeders to cull poor purebreds, and thus rid the herd registers of undesirable blood-lines rather than to continue passing them on to lower-class herds as registered animals, there to multiply their kind.

TABLE 4

Average Advanced Registry records on Guernsey

YEAR	MILK	BUTTERFAT PERCENTAGE	BUTTERFAT
1920	8,956	5.17	463
1921	9,144	5.35	488
1922	9,052	5.35	484
1923	9,262	5.43	503
1924	9,741	5.44	529
1925	10,037	5.41	542

The Advanced Registry must not be overlooked as an instrument of improvement in the dairy cattle industry. All of the European breed associations that have made definite progress, have accomplished it through two avenues, namely: (*a*) by reducing the numbers of cull purebreds from the dairy population, and (*b*) by locating and popularizing the highest class of purebreds, that these will be used to bring improvement among Class II registered cows, and to maintain the high standards among registered cows in Class I.

REFERENCES

(1) Hoxie, Jane L. 1923 Solomon Hoxie, a biography by his daughter. J. J. Little & Ives Co., New York. Pages 67, 97.

(2) Hill, C. L. 1917 The Guernsey Breed. Fred L. Kimball Co., Waterloo, Iowa. Pages 37–38; 162–165.

(3) Coates, George 1822 The general Short-Horned Herd Book: Containing the pedigrees of Short-Horned bulls, cows, etc., of the improved Durham breed, from the earliest account to the year 1822. Wharfdale Stanhope Press. Pages 68–69.

(4) Hoxie, Solomon 1882 Main or Advanced Register. Dutch-Friesian Herd Book, ii, 165–166.

(5) Hoxie, Solomon 1886 Advanced Register—its general designs. Holstein-Friesian Herd Book. Bulls. ii, 34–37.

(6) Gow, R. M. 1909 Tests of Jersey cows. Their reception and development by the American Jersey Cattle Club. Jersey Sires and their Tested Daughters, Pages v–xvl.

.... 1927 Register of Merit testing. American Jersey Cattle Club pamphlet, 3.

(7) Babcock, S. M. 1890 A new method for the estimation of fat in milk, especially adapted to creameries and cheese factories. Wis. Agr. Exp. Sta. Bul. 24.

(8) .... 1894 Advanced Register rules. Holstein-Friesian Herd Book, xii, 84, 756.

(9) Eckles, C. H. 1926 History and trends of official testing. The Jersey Bulletin, xlv, 2099–2100, 2132, 2143–2144, 2172.

(10) Rabild, Helmer 1911 Cow testing associations. U. S. Dept. Agr. B. A. I. Circ. 179, 5–13.

(11) .... 1928 Report on milk records. Scottish Milk Records Association, 63–81, 83.

(12) Conklin, C. T., and C. L. Burlingham 1928 Information to the authors.

(13) Gardner, M. H. 1928 Rules and regulations for the conduct of Herd Improvement tests of Holstein-Friesian cows. Holstein-Friesian Association of America, Rule 6, 4.

(14) Morley, Lewis W. 1928 A. J. C. C. organizes Herd Improvement. The Jersey Bulletin, xlvii, 685–686.

(15) .... 1927 Rules for the conduct of official tests recommended by the American Dairy Science Association. Hand-Book of Rules and Regulations for the Conduct of Official Tests of Holstein-Friesian Cows. Pages 10–15.

(16) Becker, R. B. 1925 Calculating the average production of a dairy herd. Jour. Dairy Sci., viii, 105–114.

(17) .... Rules for registration of purebred dairy cattle of the several breeds.

(18) McDowell, J. C. 1927 Testing cows for production every other month. U. S. Dept. Agr. Circ. 1, 1-3.

(19) Copeland, Lynn 1928 Monthly and bi-monthly tests. The Jersey Bulletin, xlvii, 731-732.

(20) Petersen, Wm. E. 1925. Accuracy of one-day and two-day tests. The Jersey Bulletin, xliv, 2044, 2058.

Petersen, Wm. E. 1925 One way of lowering the cost of records. Holstein-Friesian World, xxii, 1944-1945, 1950.

(21) Regan, W. M., and S. W. Mead 1921 Factors affecting the total butterfat content of cow's milk during a period of two days. Jour. Dairy Sci., iv, 495-509.

(22) Fitch, J. B., R. B. Becker, and P. C. McGilliard. 1922 Is a preliminary dry milking essential in semi-official tests? Jour. Dairy Sci., v, 259-272.

(23) Wylie, C. Elmer 1923 Factors influencing two-day official butterfat tests of cows. Jour. Dairy Sci., vi, 292-298.

(24) Brintnall, Earle 1924 The official test in the South and the preliminary milking. Jour Dairy Sci., vii, 393-402.

(25) .... 1928 Holstein-Friesian Association meets. Hoard's Dairyman, lxxiii, 608.

(26) Norton, H. W., Jr. 1928 Herd Improvement Registry. Holstein-Friesian World, xxv, 1328, 1330, 1332.

(27) .... 1926 The Ayrshire Digest, x, no. 9, 9.

(28) McDowell, J. C., and J. B. Parker 1926 Better cows from better sires. U. S. Dept. Agr. Dept. Circ. 368, 1-14.

(29) Fairchild, L. H. 1926 Good bulls and good foundation cows, the source of better dairy cattle. Purdue Univ. Bul. 299, 3-20.

(30) Hooper, J. J. 1924 History of the Kentucky Jersey herd. The Jersey Bulletin, xliii, 359, 366.

(31) Copeland, Lynn 1927 Inheritance of butterfat percentage in Jersey cows. Jour. Dairy Sci., x, 344-352.

(32) Burrington, W. D., and G. C. White 1925 Inheritance of the per cent of fat in a Holstein herd. Jour. Dairy Sci., viii, 215-229.

(33) Faber, H. 1921 Improvement of dairy cattle in Denmark. Jour. Min. Agr. (Gr. Brit.), xxviii, 598-607, 704-711.

(34) Savage, John M. 1927 Results of enforced selection. Guer. Br. Jour., xxxii, 644.

(35) Anthony, E. L. 1928 Lifting a breed by the herd-test. Breeder's Gazette, xciii, no. 4, 58-61. The Ayrshire Digest, xiv, no. 5, 6-7.

(36) Glover, A. J. 1928 Making dreams come true. Holstein-Friesian World. xxv, 1173-1188.

THE MOLD AND YEAST COUNT AS AN INDEX OF THE KEEPING QUALITY OF BUTTER*

H. MACY AND H. B. RICHIE

Dairy Bacteriology Laboratory, University of Minnesota, St. Paul, Minnesota

When the mold and yeast count of butter was introduced in Canada and this country a few years ago there was a hope expressed that such an analysis might prove a valuable aid in foretelling the keeping quality of butter in storage. A number of people have held the opinion that it should serve as an index of keeping quality as well as a criterion of pasteurization efficiency and sanitation in the creamery. No extensive data have been offered to prove or disprove this contention. The Dairy Division at the University of Minnesota has availed itself of an opportunity to study this problem. At the time of the 1927 contest of the National Creamery Buttermakers' Association, the butters to be placed in storage were scored at the University and likewise when they were withdrawn. This provided for analysis nearly 300 samples of butter from many different localities and which were manufactured with great care and stored under commercial cold storage conditions. It has also been possible for us to obtain representative samples of print butter from a number of Minnesota and Wisconsin creameries. The deterioration of print butter has not been followed closely, especially when stored under temperatures often prevailing in refrigerator cars, market warehouses and refrigerators in retail establishments or homes. Consequently, there was an opportunity to correlate yeast and mold counts with the deterioration of such butter kept at temperatures much higher than those which prevail in cold storage warehouses and for shorter periods of time.

---

* Published with the approval of the Director as paper 824, Journal series of the Minnesota Experiment Station. Received for publication December 31, 1928.

REVIEW OF LITERATURE

During the past ten years a number of papers have appeared in dairy literature concerning the mold and yeast counts of butter. Lund (8, 9, 10, 11, 12, 13, 14, 15, 16) has contributed widely in this respect and offers data for a large number of samples with especial emphasis upon the importance of these counts in determining the efficiency of pasteurization and plant sanitation. These articles have been accompanied by those of Bouska and Brown (3), North and Reddish (18), Redfield (20), Stiritz (22), Gregory (4), Parfitt (19), Shutt (21), Hood and White (5, 6), Johns (7), and Macy (17). All of these authors have clearly expressed the opinion that the mold and yeast count of butter is a useful index of the efficiency of creamery operations, especially of pasteurization and sanitation.

Parfitt (19) reports that he did not find any apparent relationship between the mold count and the score of butter but did note that the butters with lower scores showed higher yeast counts. The samples which he studied, however, were poorer grades of butter. Redfield (20) states that in general the lower grades of butter which he studied showed high mold and yeast counts. Gregory (4) found that 66.7 per cent of the mold counts and 23.9 per cent of the yeast counts of 94 scoring butter entered in the 1926 contest at the National Dairy Exposition were below 50 per cubic centimeter.

In 1913 Bouska and Brown (3) considered the possibility that the keeping quality of butter might be foretold by determining the mold and yeast content. They were successful in a certain percentage of the cases but remarked that, "Our way selected so few good keepers that a cold storage could not have made a living by our method." Lund (12, 13, 14) considered in 1921 that no relationship between the yeast count of butter and its flavor or keeping quality could be shown from the evidence then at hand. Shutt (21) concluded from a study of 21 samples stored at 21°F. for 6 months that apparently butter made from cream that was thoroughly pasteurized and protected as much as possible from recontamination would maintain a keeping quality superior to butter made from cream contaminated during churning. Gregory (4) makes the statement that "It is not surprising to see no relationship existing between the keeping quality and the yeasts and molds" in the butter he examined. Hood and White (4) are of the opinion that butter made from cream inefficiently pasteurized and recontaminated in handling and containing a large number of molds and yeasts, was subject to molding before being consumed.

From a perusal of this literature it is apparent that there is some difference of opinion as to the value of the mold and yeast count as an index of keeping quality. There is also a dearth of experimental data upon the subject.

SOURCE OF MATERIAL

The samples of butter which served as the bases for the studies reported herewith will be considered in four groups which are explained as follows:

Group I. The entries in the 1927 storage butter contest of the National Creamery Buttermakers' Association consisted of 297 lots of butter, most of which were in twenty pound wooden tubs. These samples of butter were available for analysis at the time they were given their original score in June by three nationally known judges. A sample was taken from each tub at that time and a mold and yeast count made therefrom. These butters were removed to a commercial cold storage warehouse where they were stored for five months at −5° to −10°F. At the time they were withdrawn from storage another scoring was made by the same judges so that it was possible to determine the keeping qualities of each piece of butter and compare it with the original mold and yeast count.

Group II. This group consisted of 100 prints of butter purchased locally and representing fresh butter from 100 different creameries in Minnesota and Wisconsin. These prints were quarter pounds, half pounds, or pounds but all were in one pound cartons. They were scored by official Federal-State Butter Graders at the time they were purchased and after they had been stored for 6 weeks at 34° to 36°F. in the cooler of the creamery at the University Farm.

Group III. This set of butters was purchased at the same time as Group II and represented the same creameries. This group was scored as above, both fresh and after storage at 50° to 52°F. for 6 weeks in a laboratory refrigerator.

Group IV. One hundred lots of print butter were purchased a year later from the same source as Groups II and III. In all there were five hundred pounds purchased, five pounds from the same churning from each of 100 creameries. The prints were selected from the printing tables in sequence so that they might be reasonably comparable in quality. Each lot was scored by the official graders when obtained. Analyses were made of one sample of each lot when fresh. One of the remaining prints of each lot was then stored for 4 weeks, another lot for 6 weeks, at 34° to 36°F. in the cooler at the University Farm, while the two remaining prints were placed in the laboratory refrigerator at 50° to 52°F. for periods of 4 and 6 weeks respectively. At the end of these storage periods all samples were rescored by the official graders.

METHODS OF ANALYSIS

Sampling

Group I. A butter trier was used to obtain a 4-inch plug of butter from the tubs to be analyzed. This sample was then placed, with the aid of a sterile wooden spatula, in a sterile glass jar provided with an aluminum screw top.

Groups II and III. Slices of butter were cut from the ends of the prints by means of a sterile wooden spatula and placed in glass jars as above.

Group IV. One-half of the pound of butter was cut into pieces and placed in a sterile, wide-mouthed, pint Mason jar with the aid of a wooden spatula.

Plating procedure

The sample jars were placed in a water bath at a temperature of 37° to 38°C. After the sample had been thoroughly melted and mixed, 1 cc. of the butter, or necessary dilutions of it, were plated as usual. The plates were poured with whey agar. Just before pouring, 1 cc. of 1 per cent sterile tartaric acid solution was added to each plate. The plates were incubated at room temperature (20° to 25°C.) for three days before counting. Counts are reported per cubic centimeter of butter.

TABLE 1

Distribution of counts in fresh butter

COUNTS	DISTRIBUTION OF COUNTS			
	Group I	Group II	Group III	Group IV
Molds per cubic centimeter	*per cent*	*per cent*	*per cent*	*per cent*
0–5	56.5	50.0	45.0	64.0
6–10	14.5	14 0	19.0	16.0
11–50	20.2	32.0	29.0	16.0
51–100	2.4	1.0	5.0	2.0
101–1,000	5.1	3 0	2.0	1.0
1,100–5,000	1.3	0.0	0.0	1.0
Yeasts per cubic centimeter				
0–5	19.2	6 0	7.0	7.0
6–10	7.4	7.0	7.0	3.0
11–50	24.7	26.0	24.0	17.0
51–100	9.5	19.0	21.0	17.0
101–1,000	25.0	42 0	41.0	39.0
1,100–5,000	9.1	0.0	0.0	13.0
5,100–25,000	3.7	0.0	0.0	4.0
25,100–90,000	1.4	0.0	0.0	0.0
Total counts				
0–5	13.2	5.0	5.0	4.0
6–10	9.1	6.0	7.0	4.0
11–50	25.0	23 0	19.0	18.0
51–100	10.5	20.0	23.0	17.0
101–1,000	27.4	46.0	46.0	40.0
1,100–5,000	9.4	0.0	0.0	12.0
5,100–25,000	4.0	0.0	0.0	5.0
25,100–100,000	1.4	0.0	0 0	0.0

PRESENTATION OF DATA

The mold counts obtained in the analysis of the fresh butters in the four groups just before they were placed into storage are given in table 1. It will be noted that more than 60 per cent of the samples contained less than 10 molds per cubic centimeter. The maximum mold count obtained did not exceed 5,000 per cubic centimeter. The majority of samples would be classed as good from the standpoint of the accepted standards of mold counts of butter.

As usual, the yeast counts were found to be higher than the mold counts, as shown also in table 1. The larger percentage of samples exhibit yeast counts above 50 per cubic centimeter. The maximum yeast count was 90,000 per cubic centimeter.

When the mold and yeast counts are combined to give the customary total count the results are as indicated in table 1.

TABLE 2

Relation between mold counts and score of fresh butter

ORIGINAL SCORE	NUMBER OF CASES	SAMPLES IN EACH GRADE SHOWING MOLD COUNTS OF 50 OR LESS PER CUBIC CENTIMETER				
		Group I	Group II	Group III	Group IV	Average for all groups
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
94.00 or higher	45	87.9	0.0	0.0	100.0	86.7
93.00–93.83	297	92.6	97.9	97.9	97.0	95.3
92.00–92.83	134	90.8	95.7	91.3	91.7	91.8
91.00–91.83	69	91.4	100.0	93.8	100.0	94.2
90.83 or lower	52	88.2	92.3	84.6	88.9	88.5
Total..........	597					

Here it will be noted that less than 25 per cent of the total counts are below 10 per cubic centimeters. In Group IV only 8 per cent of the samples have such a low count. The majority of total counts are above 50 per cubic centimeter and reach a maximum of 90,400. The distribution of these counts is sufficiently wide to permit some reasonably representative comparison.

It is interesting to note whether or not there is any correlation between the score of the butter and the mold and yeast counts. Table 2 shows the distribution of samples according to the original score. Those showing scores of 94.00 or higher are considered as one grade, those between 93.00 and 93.83 as another and so on, down to the lowest scoring butters of which there were only 7 below 90.00. The majority of samples were of 92 or 93 grade as the table points out clearly. In order to condense the material, the comparisons are made upon the basis of the percentage of samples showing mold counts of 50 or less per cubic centimeter. As an example, the table shows that 87.9 per cent of the samples in Group I, that scored 94.00 or higher, contained 50 molds or less per cubic centimeter. If the data are studied carefully there appears to be no consistent relationship between the original score of the butter and the mold counts.

The same sort of comparison may be made between the yeast, or total count and the score of the butter. Likewise, there is no apparent relationship between yeast or total count and original score.

The question is sometimes raised as to whether or not high yeast counts accompany high mold counts. When the ten samples showing the highest mold counts in each group were selected, it was found that 80 per cent of the samples in Group I and 90 per cent in Groups II, III, IV had accompanying yeast counts which were more than 100 per cubic centimeter. In the same way, when the samples showing the ten lowest mold counts were selected, it was noted that only 20 per cent of the samples in Group I, none in Group II, 10 per cent in Group III and 40 per cent in Group IV had accompanying yeast counts above 100 per cubic centimeter. Apparently there was a tendency for high yeast counts to accompany high mold counts in these samples of butter.

When one reaches the point where an attempt is made to determine whether or not there is any correlation between the mold or yeast count and the keeping quality of the butter he is confronted with the selection of the proper basis of comparison. Where an average of the individual losses in score is made it has a tendency to blend the high and low points so that the end result does not always represent the true situation. However, table 3 gives the average loss in score which occurred in samples of varying mold and yeast counts in the four groups. It will be noted that there are considerable fluctuations in the average losses of samples of varying mold or yeast content. It may be noted that there is a slight tendency for the samples with the lower counts to show less deterioration than those with high counts. This would be brought out more clearly if the samples were combined into those containing fifty or less molds or yeasts per cc. and those containing more than that. If this were done, the only exception would prove to be among the samples in Group IV stored for 4 weeks at 50° to 52°F. In any case, however, the differences are so slight that they are scarcely significant. The difference of opinion which might exist between three judges scoring the same sample of butter might very easily be greater than the differences indicated by the average figures.

TABLE 3

Relation between mold, yeast, and total counts and keeping quality of butter

ORIGINAL COUNT	AVERAGE LOSS IN SCORE DURING STORAGE						
	Group I, − 5 to − 10 °F., 5 months	Group II, 34–36 °F., 6 weeks	Group III, 50–52°F., 6 weeks	Group IV			
				34–36°F., 4 weeks	50–52°F., 4 weeks	34–36°F., 6 weeks	50–52°F., 6 weeks
Molds per cubic centimeter							
0–10	−0.49	−2.81	−3.63	−2.69	−3.13	−3.73	−4.38
11–50	−0.60	−3.29	−4.59	−3.16	−3.78	−3.53	−4.50
51–100	−0.63	−2.00	−5.30	−4.00	−3.75	−4.25	−4.25
101 or more	−1.15	−4.50	−3.50	−4.00	−3.00	−3.00	−3.50
Yeasts per cubic centimeter							
0–10	−0.57	−2.92	−3.54	−2.20	−2.35	−3.15	−4.05
11–50	−0.34	−2.73	−3.81	−2.85	−2.76	−3.85	−4 12
51–100	−0.71	−2.92	−3.50	−2.62	−3.53	−3.76	−4.71
101 or more	−0.68	−3.29	−4.49	−2.98	−3.46	−3.72	−4.41
Total per cubic centimeter							
0–10	−0.54	−2.91	−3.55	−2.13	−2.44	−3.06	−4.19
11–50	−0.35	−2.65	−3.76	−2.75	−2 72	−3.81	−4.17
51–100	−0.49	−2 93	−3 67	−2.68	−3.47	−3.76	−4.47
101 or more	−0.72	−3.27	−4.35	−2.98	−3.45	−3.73	−4.44

It is interesting to note in table 3, the marked deterioration of the print butter in Groups II, III, and IV, at the temperatures of the better household and grocery refrigerators, refrigerator cars or even the cool rooms of the warehouses of wholesalers and distributors. Even though these samples have been stored somewhat longer than the average print of butter is kept, it is conceivable, and in fact it has been shown, that some prints are held for such periods of time before the dealer disposes of them. Further they are seldom stored at temperatures below 30°F. from the time they leave the creameries or western warehouses, until they reach the consumer's table. The importance of temperature in the deterioration of butter is clearly demonstrated by these data.

If one considers the relation between the counts and the deterioration of the butter from another angle, he might determine whether there was a greater tendency for high count butters to lose in score during storage by figuring the percentage of samples with different counts which deteriorated. For example, it was noted that while 63.9 per cent of the samples in Group I containing 10 or less molds per cubic centimeter showed a loss in score during storage, 84.2 per cent of the samples with a count of over 100 per cubic centimeter deteriorated. This shows a tendency toward better keeping quality in the low count butter. In general this appears to hold true according to the data for all groups but the difference is so comparatively small, it is almost insignificant.

There is still another method for presenting the data concerning the relation of counts to keeping quality. This is shown in Figure 1 in which distribution curves are presented. As will be noted, there are two curves, one for the samples showing a total count of 50 or less per cubic centimeter and the other for those with more than 50. The percentage of samples in each category showing the various losses or gains in score is illustrated in the graph. The curves are typical of those which might also be presented for mold or yeast counts in any one of the four groups but which have been omitted in order to conserve space. As has been indicated in table 3, there is a slight advantage in keeping quality to be recorded for the low count samples but it is exceedingly meagre.

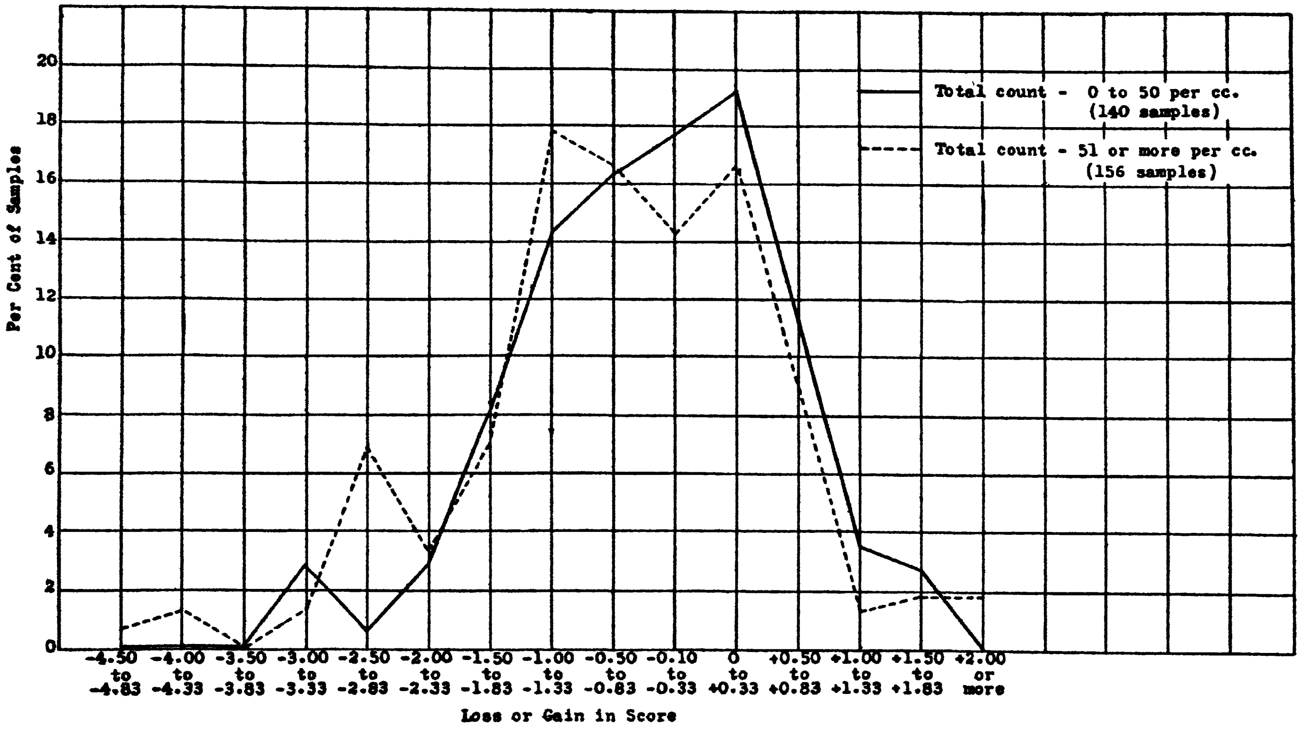


FIG. 1. DISTRIBUTION OF SAMPLES UPON BASIS OF TOTAL COUNT AND GAIN OR LOSS IN SCORE DURING STORAGE

Data based upon results from 297 samples in Group I stored for 5 months at −5 to −10°F. This curve represents the situation with respect to other counts and groups.

Correlation tables have been prepared from the data reported but inasmuch as the methods of presentation of the data, which have been used above, give the results in essentially the same relationships, they have also been omitted. They show no real correlation between the counts and keeping quality.

TABLE 4

Mold, yeast, and total counts as indices of keeping quality

Data from results obtained in Group I stored for 5 months at −5 to−10°F. They are representative of data in other groups.

MOLD COUNTS	GAIN OR LOSS IN SCORE	YEAST COUNTS	GAIN OR LOSS IN SCORE	TOTAL COUNTS	GAIN OR LOSS IN SCORE
Minimum per cubic centimeter		Minimum per cubic centimeter		Minimum per cubic centimeter	
0	−4.50	0	−3.34	0	−2.00
0	−3.34	0	−2.50	0	−1.50
0	−2.50	0	−1.50	0	−1.34
0	−2.00	0	−1.50	0	−0.16
0	−2.00	0	−1.50	0	+0.34
0	−1.84	0	−1.16	1	−1.50
0	−1.75	0	−0.84	1	−0.50
0	−1.50	0	−0.50	1	−0.34
0	−1.34	0	−0.34	1	0
0	−1.34	0	−0.34	1	+0.34
Maximum per cubic centimeter		Maximum per cubic centimeter		Maximum per cubic centimeter	
3,140	−0.50	90,000	−2.50	90,400	−2.50
2,600	−0.50	90,000	+0.34	90,130	+0.34
2,250	−1.84	44,000	−1.50	44,140	−1.50
1,140	−2.75	30,000	−0.66	30,450	−0.66
900	+0.84	24,000	−2.34	24,310	−2.34
800	−1.00	24,000	−1.66	24,016	−1.66
570	+0.16	12,000	+0.16	12,600	−0.50
480	−0.50	11,200	−1.09	12,520	+0.17
450	−0.66	11,000	−0.16	11,200	−1.09
420	−1.00	10,000	−0.50	11,050	−0.16

To approach the situation from a different angle, consider the possibility that there was a tendency for the low count samples to show better keeping quality such as indicated by the averages in table 3. Then one may select samples from among those showing high or low counts and determine whether it would be possible to pick out the samples of superior keeping quality. Such a plan is illustrated in table 4.

Ten samples in Group I showing the minimum mold, yeast and total counts were selected. The losses or gains in score accompanying these samples are likewise recorded. The same is done for the ten samples showing the maximum counts. It is readily seen that one could go far wrong in attempting to pick a lot of butter of superior keeping quality on the basis of low mold or yeast count. For example, there is a sample with a 0 mold count which lost 4.50 points in storage, while another butter

TABLE 5

Relation between maximum gains or losses in score and mold, yeast, and total counts

Data from results obtained in Group I stored for 5 months at −5 to −10°F They are representative of data in other groups

CHANGE IN SCORE	MOLD COUNTS PER CUBIC CENTIMETER	YEAST COUNTS PER CUBIC CENTIMETER	TOTAL COUNTS PER CUBIC CENTIMETER
Maximum losses			
−4.50	0	220	220
−4.00	4	74	78
−4.00	21	250	271
−3.34	0	2	2
−3.34	2	330	332
−3.34	230	300	530
−3.34	5	1	6
−3.00	4	39	43
−3.00	10	8	18
−2.84	4	665	669
Maximum gains			
+2.16	2	820	822
+2.16	21	120	141
+2.00	10	530	540
+1.84	2	350	352
+1.84	21	20	41
+1.75	9	1,300	1,309
+1.66	0	1	1
+1.66	1	1	2
+1.66	2	37	39
+1.50	3	260	263

with the very highest mold count lost only one-half point. The same sort of comparison could be made upon the basis of yeast or total counts. Obviously the mold or yeast count does not predict the keeping quality infallibly.

Table 5 carries the application one step further. Here are arranged the greatest losses and gains in score along with the counts of the butters constituting Group I. It is seen clearly that there is very little difference between the counts in either group. In other words, deterioration is not measured by the mold or yeast count.

DISCUSSION OF RESULTS

A study of the 597 lots of butter which constitute the basis for these investigations indicates that there is a wide range of mold and yeast counts among these samples which are quite representative of commercial butter. The yeast counts in general run much higher than the mold counts, which is according to the experience of others.

There does not appear to be any relationship between the counts and the score of the butter. The score of fresh butter depends upon many factors. Often the lower scoring butters are made from cream as efficiently pasteurized as that for high scoring butters. Again, it is known that the highest scoring butters, especially in contests, carry a low salt content which may explain why many of them show high counts. If total counts are considered, the averages for all the groups of samples here reported show that the lowest counts occur most frequently in the 92 grade of butter and that percentages of low counts decrease in both directions from this class. Other studies have shown that 92 scoring butter often shows the superior keeping quality. At best, however, the differences are too slight to form a basis for selection or discrimination.

While the data which are presented do not appear to show that the mold and yeast counts are of any value as indices of keeping quality, one must not infer that the determination of these counts is a useless operation. It is generally agreed that mold and yeast counts do serve a very important function in controlling manufacturing practices. They offer a means for checking the efficiency of pasteurization, sterilization of equipment, and general sanitation within the creamery. We have no substitute for them in this connection. Just what relationship yeasts and molds themselves may have to the deterioration of butter is not entirely known. It is evident however that the relationship need not be quantitative. There are many examples in microbiology where the alterations in an organic material are not a matter of numbers of microörganisms but rather of the types and their specific capabilities. Just because the mold and yeast counts do not serve as criteria of keeping quality there is no reason to assume that molds or yeasts do not play a part, directly or indirectly, in the deterioration of butter in storage. There is enough evidence available to show that certain molds are capable of bringing about marked changes in the flavor and aroma of butter. The very glaring defects in technique and the possibilities of missing foci of infection when sampling butter might often explain why defects appeared where analyses did not indicate any danger. Whatever the explanation for butter deterioration may be, it is evident that the mold and yeast counts of the butters constituting the basis for this report, did not offer any encouragement for their use as criteria of keeping quality. There are so many factors playing a part in the production or destruction of favorable characteristics of butter that it is not all unexpected that this conclusion should be reached. The mold and yeast count has an important place in the creamery industry but apparently it is not to serve as an index of the keeping quality of butter.

SUMMARY

1. Data are presented upon the mold and yeast counts and the keeping quality of 597 samples of butter. Among these 297 were in tubs and 300 in one-pound cartons.
2. The mold counts ranged from 0 to 4800 per cubic centimeter; the yeast counts from 0 to 90,000 per cubic centimeter; and the total counts from 0 to 90,400 per cubic centimeter.

3. No consistent relationship was apparent between the mold or yeast counts and the quality of the fresh butter.

4. In general, high yeast counts accompanied high mold counts.

5. Considered as a group, the samples of butter with the lower mold, yeast and total counts showed a tendency toward slightly better keeping quality than those with higher counts.

6. The mold, yeast or total count of individual samples did not serve as a reliable index of the keeping quality of the butter.

REFERENCES

(1) Abbott, F. H.: Standardization and improvement of California butter. California Exp. Sta. Bul. No. 443 (1927).

(2) Anon.: Manufactured products. Indiana Sta. Rpt. 1926, pp. 26, 27 (1926).

(3) Bouska, F. W., and Brown, J. C.: The significance of yeasts and Oidia in pasteurized butter. Wis. Univ. Studies, Sci. No. 2, pp. 131–8 (1921).

(4) Gregory, H. W.: Study of exposition butter. Butter, Cheese, and Egg Jour., Feb. (mo. ed.), pp. 42, 44, 46, 48 (1927).

(5) Hood, E. G., and White, A. H.: The cause and prevention of mould in Canadian pasteurized butter. Dominion of Canada, Dept. of Agr. Bul. No. 48, new series. Ottawa (1925).

(6) Hood, E. G., and White, A. H.: Studies on moulds and yeasts in creamery butter. Dominion of Canada, Dept. of Agr. Pamphlet No. 92. New series. Ottawa (1928).

(7) Johns, C. K.: A rapid method for determining the mold and yeast count of butter. Sci. Agr., viii, 353–69 (1928).

(8) Lund, T. H.: Yeasts and moulds in butter. 44th Ann. Rpt. Ontario Agr. College, pp. 39–41 (1918).

(9) Lund, T. H.: Yeasts and moulds in butter and cream. Rept. Proc. of Dom. Dairy Conference, Ottawa. November 25–28, 1918, pp. 59–63 (1918).

(10) Lund, T. H.: Notes on yeast and mould counts of creamery butter, 1919. Dairymen's Assoc. Ontario, Ann. Rpts. pp. 90–94 (1919).

(11) Lund, T. H.: Yeasts and molds in butter. N. Y. Prod. Rev. and Amer. Cream., xlviii, 1184–92 (1920).

(12) Lund, T. H.: Yeasts in pasteurized cream butter and some suggestions as to their probable source. Abstracts Bact. v, ii (1921).

(13) Lund, T. H.: Yeasts and molds in pasteurized cream butter. N. Y. Prod. Rev. and Amer. Creamery, li, 510–11 (1921).

(14) Lund, T. H.: Yeasts in pasteurized cream butter. Creamery and Milk Plant Monthly, x, no. 1, p. 30 (1921).

(15) Lund, T. H.: Buttermakers' interest in microörganisms. N. Y. Prod. Rev. and Amer. Creamery, liii, 693–8 (1922).

(16) Lund, T. H.: Microörganisms in creamery butter. Sci. Agr., ii, 332–40 (1922).

(17) Macy, H.: Mold and yeast counts and their relation to the composition of butter. Jour. Dairy Sci., x, 384–95 (1927).

(18) North, W. R., Jr., and Reddish, G. F.: Yeasts and Oidia in high-grade experimental butter. Jour. Dairy Sci., iv, 510–20 (1921).
(19) Parfitt, E. H.: Relationship between yeasts and molds in butter and the butter score and care in manufacture. Creamery and Milk Plant Monthly, xl, no. 1, pp. 53–4 (1926).
(20) Redfield, H. W.: The determination of yeasts and Oidia in cream and butter. Jour. Dairy Sci., v, 14–21 (1922).
(21) Shutt, D. B.: The relation between yeasts and molds and the keeping quality of butter. Jour. Dairy Sci., vii, 357–60 (1924).
(22) Stiritz, B. A.: Yeast and mold counts and their relation to pasteurization of cream for buttermaking purposes. Jour. Dairy Sci., v, 362–71 (1922).

A COMPARISON OF THE BROMTHYMOL BLUE MILK TEST AND THE METHYLENE BLUE REDUCTION TEST FOR DETERMINING QUALITY OF MILK*

E. D. DEVEREUX

Michigan Agricultural Experiment Station, East Lansing, Michigan

REVIEW OF THE LITERATURE

The bromthymol blue milk test or colorimetric hydrogen ion method was devised by Cooledge (1, 2) in 1920 to determine the keeping quality of milk. The test was compared to other methods, particularly the methylene blue reduction test and the standard plate count. These comparisons were made by using correlation coefficients. In each case the correlation coefficient between the test in question and the keeping quality was determined; the results were as follows:

Bromthymol blue score (Cooledge score)	+0.75
Methylene blue reduction test	+0.52
Agar plate count	−0 25

Cooledge measured the keeping quality by the time required for the milk to show the first appearance of souring, as determined by taste. The milk was held at room temperature. One hundred fifteen samples were examined. The bromthymol blue scores were made according to the method as devised by Cooledge (see experimental); the methylene blue reduction test was applied as described by Hastings (3); the plate counts were made according to "Standard Methods" (1919).

Ellenberger et al. (5) made a comparison of the methylene blue reduction test and the agar plate count for determining the quality of milk. The keeping quality (time) of 268 samples was determined. The correlation coefficients were as follows:

Methylene blue reduction test	+0.72	±0.02
Agar plate count	−0.28	±0.04

---

* Received for publication January 11, 1929. Paper No. 3 (n.s.) from the Michigan State College Agricultural Experiment Station.

Ellenberger et al. measured the keeping quality by the time required for the milk to become unfit for table use as determined by taste and acid development. The bacteriological examinations were made according to "Standard Methods."

EXPERIMENTAL

To the knowledge of the author the studies of Cooledge have never been repeated, and, since the work of Ellenberger, et al. on the reduction test did not corroborate the results secured by Cooledge, it was desired by the author to repeat some of this work.

In the experimental work the bromthymol blue test and the methylene blue reduction test were compared, using the actual keeping quality (time) as the standard.

One hundred fifty 200-cc. samples were taken in 250-cc. sterile Erlenmeyer flasks from the receiving vats of two dairies.

The bromthymol blue test was performed as devised by Cooledge with the exception that a slight change (6) has been made in the formula of the Cooledge broth. The test is made by adding 0.1 cc. of the milk in question to 10 cc. of bromthymol blue broth and noting the rate of pH change when incubated at 37°C. until a pH of 5.8 is reached or until the tube has been incubated for eight hours.

Formula of Cooledge broth[1]

Beef extract	3 grams
Peptone	10 grams
Sodium chloride	5 grams
Dibromthymosulphonephthalein (Bromthymol blue)	0 020[2] grams
Distilled water	1000 cc.

Adjusted to pH = 7. Autoclaved at 15 pounds for fifteen minutes.

Bromthymol blue color standards[3] having a pH range of 5.8 to 7.4 (5.8, 6.0, 6.2,. . ., 7.4) are used for making the readings.

---

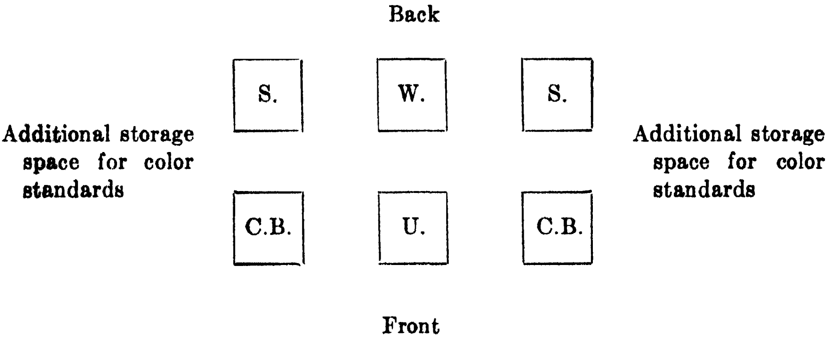
[1] This is the formula that has been recommended to the Digestive Ferments Company, Detroit.

[2] Recently changed from 0.032 to 0.020 gram per liter.

[3] The test has been so devised that the color standards and comparators of either the Hynson, Westcott, Dunning Company, (use test tubes with a diameter of 1.8 cm.) or the LaMotte Chemical products Company, can be employed (use tubes with a diameter of 1.5 cm.).

Broth without the indicator is also prepared and used in the compensating blanks. The compensating blanks are inoculated and incubated in the same manner as the broth tubes containing the indicator. It is necessary to prepare only two compensating blanks for one day's run or any one batch of samples.

When it is desired to make a reading, the tubes and the compensating blanks are removed from the incubator, cooled to 20°C., and placed in the comparator. The top view of the arrangement of the tubes in the comparator when a reading is to be made is given below.


Back
S.
W.
S.
Additional storage space for color standards
Additional storage space for color standards
C.B.
U.
C.B.
Front

S. = color standard
W. = water blank
C.B. = compensating blank
U. = unknown containing the milk in question

The unknown is compared to the color standards and the pH noted.

Cooledge devised a table in order that the milk could be scored, its keeping quality determined, and the purpose for which it is best suited determined. From experience with this table the author is of the opinion that milk scoring 70 or better is suitable for milk plant supply

The methylene blue reduction test was made according to "Standard Methods" (fifth edition, 1927), using approved standardized tablets from which a fresh solution was prepared every few days.

The keeping quality was measured by the time required for the milk to become unfit for table use as determined by taste. The samples were held at 18° to 20°C.

TABLE 1

The comparison of the results secured by the bromthymol blue and methylene blue tests and the keeping quality of milk

TIME OF SOURING	NUMBER OF SAMPLES	BROMTHYMOL BLUE TEST			METHYLENE BLUE REDUCTION TEST			
		Score range*	Average score	Number of exceptions	Reduction range†	Average reduction time	Milk class†	Number of exceptions
hours					*minutes*	*minutes*		
8	2	35–55	37.5	0	0–20	3	Very bad	0
12	9	35–55	44.4	0	20–120	57	Bad	3
18	31	35–55	50.2	2	120–330	123	Fair	18
20	16	35–55	51.2	2	120–330	228	Fair	7
22	8	60–75	58.7	8	120–330	180	Fair	5
24	8	60–75	59.4	8	120–330	218	Fair	4
26	14	60–75	65.0	12	120–330	317	Fair	4
28	13	60–75	71.0	9	120–330	289	Fair	6
30	13	60–75	70.5	12	330+	342	Good	8
32	14	80–100	85.0	2	330+	424	Good	3
34	10	80–100	85.0	2	330+	413	Good	2
36	8	80–100	89.4	2	330+	466	Good	2
38	2	80–100	90.0	0	330+	585	Good	0
44	2	80–100	95.0	0	330+	525	Good	0
	150			59				62

* Score range:

READING	pH	SCORE	MILK SUITED FOR
hours			
1, 2, 3	5.8	20–30	Condemning
4, 5, 6, 7, 8	5.8	35–55	For butter making
8	5.9–6.2	60–75	Condensing
8	6.3–6.7	80–100	Market milk or cheese making

† "Standard Methods" classification of milk according to reduction time:

Class 1—good milk, not decolorized in 5.5 hours
Class 2—fair milk, decolorized in 2 to 5.5 hours
Class 3—bad milk, decolorized in 20 minutes to 2 hours
Class 4—very bad milk, decolorized in less than 20 minutes

In table 1 the results are arranged according to the time required for the samples to become unfit for use. Since souring was, in the great majority of the cases, the first undesirable quality displayed, the column is headed "Time of souring."

An examination of the table shows that as the time of souring increased there was a steady increase, with two exceptions, in the bromthymol blue average scores. In one of the exceptions the score dropped 0.5 of a point as the time of souring increased two hours, while in the other case the average score remained stationary. The average reduction times show more fluctuations than the other scores, but it will be noted that, if these average reduction times are interpreted in terms of "milk classes," there is a steady increase in the classes as the time of souring increases.

The average score or reduction time for each group of samples determined the score range or reduction time range for that group, as given in table 1 with footnotes. This permitted the detection of those samples whose score or reduction time did not fall in the determined range. These cases were counted and the number in each group recorded under the column heading "Number of exceptions."

TABLE 2

The correlation coefficients of the colorimetric tests and the keeping quality of milk secured by three investigators

METHOD	COOLEDGE'S COEFFICIENTS	ELLENBERBER'S (ET AL.) COEFFICIENT	THIS AUTHOR'S COEFFICIENTS
Bromthymol blue test	+0 75	No experimental work	+0 77
Reduction test..............	+0 52	+0 72 ±0 02	+0.77

Comparing the two tests from the standpoint of exceptions both seem to have about the same accuracies in detecting the poor milk and the good milk, and about the same inaccuracies in regard to the intermediate grade.

The individual cases, that is the score and reduction time of each sample (data too voluminous to present), were used to calculate the correlation coefficients between the two methods and the keeping quality of milk, and, strange to say, were both found to be +0.77. This corroborates the work of Cooledge on the bromthymol blue test but not his work on the reduction test.

However, Ellenberger et al., as mentioned earlier, found the reduction test to have a correlation coefficient of +0.72 ±0.02 which is in accordance with the coefficient found for that method in this work. These results of the three investigations are summarized in table 2.

In reviewing the technique used by Cooledge in performing the reduction test it was found that he used one part of dry dye to 100,000 parts of milk, which is about twice the concentration of the dye when the approved tablets are used. The reduction time of methylene blue of this concentration was determined on 75 samples of milk and compared to the reduction time when the approved tablets were used. In every case when methylene blue of a concentration of one part of dry dye to 100,000 parts of milk was used the reduction time was from a few minutes to forty minutes longer than when a solution of an approved tablet was used. This may account for some of the difference in the results since the approved tablets were used in this work and the work of Ellenberger et al. However, according to Hastings (4) it should not be a great factor. He found that the methylene blue could be reduced to 1:300,000 or 1:400,000 parts of milk without materially changing the reduction time.

SUMMARY

The bromthymol blue milk test was found to fulfill its purpose in that it graded milk fairly accurately into a large number of classes according to keeping quality, when average scores were studied.

The reduction test was found to fluctuate more but was able to grade the milk into four classes equally as accurately.

The correlation coefficients between each test and the keeping quality were both found to be +0.77.

The writer wishes to express at this time his gratitude to Prof. L. C. Emmons of the Mathematics Department of Michigan State College for his assistance in interpreting these data.

REFERENCES

(1) Cooledge, L. H., and Wyant, R. W. 1920 The keeping quality of milk as judged by the colorimetric hydrogen ion determination. Jour. Dairy Sci., iii, 156–166.

(2) Cooledge, L. H. 1921 The colorimetric hydrogen ion determination as a means of studying biological changes in dairy products. Michigan Agr. Exp. Sta., Tech. Bul., No. 52.

(3) Hastings, E. G. 1919 Comparative value of quantitative and qualitative bacteriological methods as applied to milk, with special consideration of the methylene blue reduction test. Jour. Dairy Sci., ii, 293–311.

(4) Hastings, E. G. 1924 The standardization of the methylene blue reduction test for milk control work. Inter. Assoc. Dairy and Milk Inspectors, 13th Ann. Rept., 268–275.

(5) Ellenberger, H. B. et al 1927 A comparison of the methylene blue reduction test and the agar plate count for determining quality of milk. Vt. Agr. Exp. Sta., Bul. No. 264.

(6) Devereux, E. D. 1928 Test for keeping qualities of milk modified. Mich. Agr. Exp. Sta. Quar. Bul. 10, 190–194.

A SURVEY OF PRESENT METHODS OF GRADING MILK AT CONDENSERIES, EVAPORATED AND DRY MILK PLANTS

The need of definite information regarding the methods in use at the present time in this branch of the dairy industry is evident to the sub-committee on Bacteriological Methods for Condensed, Evaporated and Dried Milk of the American Dairy Science Association. By making use of mailing lists furnished by the American Dry Milk Institute and Evaporated Milk Associations addresses of the companies manufacturing the products were obtained. Through the suggestion of Dr. R. S. Breed, Chairman of the General Committee, an effort was first made to get in touch with the person in each organization that was directly in charge of the control work for each particular company. Thirty-one companies expressed their willingness to coöperate and were sent a questionnaire asking for information on the subject. From the 31 companies 20 questionnaires have been received up to the present time. Thirteen companies were manufacturing condensed, the same number evaporated, 12 spray powder, 4 roller process powder and 3 reported that they manufactured powder but failed to specify the kind manufactured.

The reports regarding the examinations of the milk as received indicate that the conventional "nose" test is still used on each can at the majority of the plants. Questionable milk is further checked by a variety of tests. Acidity determinations are used by 12 plants to check such milk, 5 used the alcohol coagulation test, while 1 used the alcohol cresol purple test, 1 the brom cresol purple test, another the alcohol methylene blue test and 1 the live steam test. The use of the sediment test was reported in 11 cases and was used at irregular intervals.

The methylene blue reductase test was used by 5 companies and one reported that it gave unsatisfactory results. Seven plants used the breed microscopic count and 3 the plate count, all of this work being done in a more or less irregular and unstandardized way.

The standards used for rejecting milk were, acidity 0.18 to 0.20 per cent, temperature over 60° to 65°F. and coagulation with alcohol test. The bacterial standards varied from 100,000 to 1,000,000 per cubic centimeter, and milk showing reduction of methylene blue in less than two and one-half hours was considered unsatisfactory.

In reporting methods of sterilizing equipment 10 used some form of hypochlorites or chlorine solution, 5 used steam, 1 hot water, 1 cleaning agents and 3 failed to report. Seven stated that they checked the sterility of the equipment. The methods used for checking sterility varied greatly. Three reported the use of sterile water rinse, 1 used the count obtained from the water run through, 1 used the count from the first milk through, while 2 failed to specify method used. The standards used varied with the method used.

In checking the finished product, 7 companies indicated that a regular check was made by the plate method. One of these also ran the American Public Health Association test for *B. coli* as given in the Standard Methods of Water Analysis, 2 reported the occasional use of the plate method and methylene blue test, 1 used incubation tests on canned goods only, and 10 failed to report. Two companies, however, stated that they were installing bacteriological laboratories in the near future.

The standards for the finished product as reported by 6 companies were as follows: all required evaporated milk to be sterile, 2 required condensed milk to have a count under 1,000 colonies per gram, 1 below 10,000, 2 below 25,000 and 1 under 100,000. Two companies reporting on milk powder required a count of less than 300,000 and 500,000 per gram.

In general it would seem that from these results that the bacteriological control of the manufacture of condensed, evaporated and powdered milk is not as well established as it should be and that there is a need for information regarding practical methods of such control. It is the hope of the committee that they may secure sufficient coöperation to be able to draft a report that will fill this need. Such a report should be of value to the industry in encouraging better control of these products.

Perhaps the most important need at the present time, is a standardization of the method of determining the count from the finished product, especially milk powders. There is a prospect that a joint study of methods for this purpose may be carried out during the coming year with the Committee on Laboratory Methods appointed by the American Dry Milk Institute.

P. A. Downs, *Chairman,*
G. C. Supplee,
M. J. Prucha.

Lincoln, Nebraska, June 2, 1928.

A COLOR DEFECT OF PROCESS CHEESE*

WALTER V. PRICE

Department of Dairy Industry, Cornell University, Ithaca, New York

A manufacturer of a brand of process cheese found that occasional batches developed an undesirable, dark color during the heating process. This color matched very closely the color of an orange with a brownish tinge or that of thymol blue in solutions of pH 2.0 to 2.4. Although the cheese in all other respects seemed to be entirely normal, it was rejected by the trade.

The product was manufactured by grinding, and mixing together, well ripened cheese of the cheddar type, and placing the mass in a steam jacketed kettle. Water, an alkaline solution of annatto color, and an emulsifying agent were added to the cheese, and the mixture was heated slowly with constant agitation. The normal color usually darkened during the last stages of the heating process.

Observations indicated that the color change was independent of the quality of the cheddar cheese used in the blending operations, since identical cheese to which the same ingredients were added did not always develop the defect.

It seemed logical to assume that the change was associated in some manner with the heating operation itself. During this process some of the moisture was lost, the amount of which varied with the temperature used and the duration of the heating period. Laboratory trials were made to determine the influence on the color of the cheese of variations in its moisture content. The results indicated that the color could be slightly deepened by reducing the moisture content of the cheese approximately 2 per cent or more. Other trials showed that the color could be noticeably darkened by raising the temperature and increasing the duration of the heating process. These treatments, however, did not produce the characteristic color defect.

---

* Received for publication January 19, 1929.

It was customary in the factory to place a new batch of cheese in the heating unit as soon as the preceding batch was removed. In this way the machine was in continuous·use throughout the day, except for the noon recess. As a result, more or less cheese gradually accumulated around the sides and edges of the kettle. As would naturally be expected, this residual cheese assumed a darker color when it was exposed to the heat of subsequent batches. The accumulation of this discolored cheese, and the fact that the defective color usually appeared after the noon hour, when most of this substance adhered to the kettle, strongly suggested that this residual cheese was responsible for the defect.

An attempt was made to reproduce this defect in the laboratory by duplicating the factory routine as nearly as possible. The cheese was allowed to remain on the sides of the kettle in which the blending and pasteurizing was accomplished as it did in factory practice. A quantity of cheese sufficient for several batches was divided into identical portions. Batch after batch was made with no appearance of the defect. The kettle was then allowed to stand idle for two hours and the cheese adhering to it from the previous batches was not removed. The next batch of cheese made in this kettle developed the defective color. A second batch acted in the same manner. The cheese which was used in making the batch was then pasteurized in the usual manner in a clean kettle and was entirely normal in its appearance. The kettle in which the defective cheese was made was then thoroughly cleaned and another portion of the same cheese was pasteurized in it. The cheese retained its normal color. In repeated trials the defect could be reproduced and corrected by following this same procedure. These facts seemed to verify the theory that the incorporation of sufficient amounts of the dried and discolored cheese which accumulated on the unwashed kettle was actually capable of producing the undesirable color.

The manufacturer who originally called this defect to the attention of this laboratory took the necessary precautions to eliminate the possibility of incorporating this residual cheese in new batches. After a two years' interval no repetition of the trouble has occurred.

COPPER IN DAIRY PRODUCTS AND ITS SOLUTION UNDER VARIOUS CONDITIONS. II*

J. MISCALL, G. W. CAVANAUGH

Department of Chemistry, Cornell University, Ithaca, New York

AND

P. P. CARODEMOS†

Clemson College, Clemson College, North Carolina

This work is a continuation of the investigation conducted by Rice and Miscall.[1]

Quam, Soloman and Hellwig[2] have published data on the copper dissolving power of milk. They find the temperature of maximum copper solubility between 85° and 90°C. They used thirty minutes as the time of exposure of the milk to the metal. They attribute the decrease in the copper solubility curve above 90°C. to the decreasing solubility of oxygen.

METHOD OF PROCEDURE

In this investigation the method of Rice and Miscall[1] was followed. This consisted of allowing 2000 cc. of milk to stand, in glass flasks, in contact with twisted strips of copper, under the conditions of the experiment for two hours. The solution was then poured off the copper and evaporated to dryness, the residue ignited in a muffle furnace and the ash dissolved in a small quantity of nitric acid. The copper was electrolized onto a platinum flag, washed thoroughly with distilled water, and re-electrolized from a weak sulfuric acid solution onto a weighed rotating platinum cathode. By using this method of electrolysis a bright, adherent deposit of pure copper is obtained which can be weighed.

The pasteurized milk used was obtained directly from the

---

* Received for publication March 4, 1929.

† Presented to the Faculty of Cornell University as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[1] Rice and Miscall, Jour. Dairy Sci., 1923, vi, 261.

[2] Quam, Soloman and Hellwig, Jour. Indust. and Eng. Chem., (1928), xx, 930.

pasteurizing apparatus of the Dairy Department at Cornell. The raw milk was obtained at the Cornell barns.

SOLUBILITY OF COPPER IN PASTEURIZED MILK

Two-liter portions of pasteurized milk were placed in 5-liter flasks. The milk was then heated to the desired temperature. As soon as the correct temperature was reached fourteen twisted, bright copper strips 1 inch wide and 5 inches long (140 square inches of surface) were placed in the flask. The milk was heated at constant temperature for two hours. The copper content of the milk was then determined. The results are tabulated in table 1.

The data are plotted in figure 1 as curve *A*.

This curve shows the same values of copper content and temperature of maximum copper solubility as obtained by Rice and Miscall.[1] The copper solubility increases up to a temperature of 140°F. and then decreases as the temperature is raised. Both increase and decrease in solubility taking place gradually.

SOLUBILITY OF COPPER IN RAW MILK

The previous experiment was repeated except that raw milk was used. The results are given in table 1.

The data are plotted in figure 1 as curve *D*. The same type of curve results as when pasteurized milk was used but the temperature of maximum copper solubility is at 150°F. It is also interesting to note that previously pasteurized milk dissolves more copper at every temperature than does raw milk.

EFFECT OF CARBON DIOXIDE AND OXYGEN ON COPPER SOLUBILITY

To determine the effect of specific gases upon the solubility of copper in milk the following experiments were performed. A 5-liter flask was stoppered with a two holed rubber stopper. Through one hole a glass tube was lead which connected the flask with a manometer. Another glass tube connected the flasks with a mercury filled, gas burette which had a two-way stopcock. The burette could be used to admit known amounts of gas into the flask and also served as a mercury pump in the usual manner. Two liters of raw milk were placed in the flask.

All the gases were exhausted from the milk, the temperature being raised to the desired value. The pumping was continued until the manometer showed a pressure equal to the vapor pressure of water at that temperature. Then the stopper in the flask was quickly removed and 14 strips of copper (140 square inches) were introduced. The admitted air was quickly pumped out. The temperature was maintained constant for two hours. Then the milk was analyzed for copper in the usual manner. The results are given in table 2.

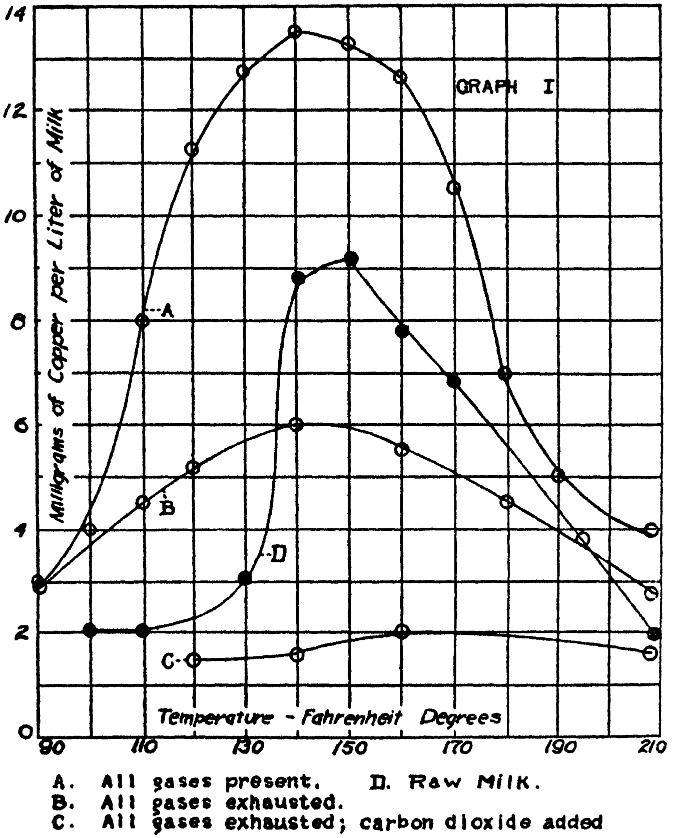


FIG. 1. SOLUBILITY OF COPPER IN MILK

The data are plotted as curve *B* in figure 1. It shows a much lower copper solubility than either the raw or pasteurized milk and a maximum copper solubility at 140°F.

TABLE 1

Solubility of copper in pasteurized milk and in raw milk

TEMPERATURE	COPPER PER LITER OF MILK	
	Pasteurized	Raw
°F.	*mgm.*	*mgm.*
70*	3.0	
100	4.0	2.0
110	8 0	2.0
120	11.3	2.5
130	12.8	3.2
140	13 5	8.8
150	13 3	9.2
160	12.7	7.8
170	10.5	6.8
180	7.0	
190	5.0	
208†	4.0	2.0

* Room temperature.
† Boiling.

TABLE 2

Solubility of copper in milk from which all gases had been exhausted

TEMPERATURE	COPPER PER LITER OF MILK
°F.	*mgm.*
70	3 0
100	
110	4 5
120	5.25
140	6.00
160	5.50
180	4.50
190.5	3.75
208	2.75

The same procedure was then followed but after all the gases were exhausted excessive amounts of carbon dioxide were added and the milk heated in contact with the copper open to the atmosphere for two hours. The results are given in table 3.

The data are plotted as curve *C* in figure 1. It shows that carbon dioxide has a definite depressing effect upon the solubility of copper in milk.

The curves show that the solubility of copper in pasteurized milk is greater than in raw milk. Both milks exhibit a maximum copper dissolving power at a definite temperature, this temperature being 5 degrees higher in raw milk than in pasteurized milk. The temperature range of maximum copper solubility is from 140° to 150°F. The copper dissolving power of milk at the boiling temperature is very near to that at room temperature. The presence of carbon dioxide greatly decreases the copper dissolving power of milk. The removal, by exhaustion, of all the gases normally dissolved in the milk also decreases the copper dissolving power of milk. Hence the presence or absence of carbon dioxide does not explain the solubility of copper in milk products.

TABLE 3

Solubility of copper in milk in which the natural gases had been replaced by carbon dioxide and oxygen

TEMPERATURE	COPPER PER LITER OF MILK	
	Carbon dioxide added	Oxygen added
°F.	*mgm.*	*mgm.*
120	1.5	11.00
140	1 55	14 00
160	2.00	13 00
208*	1.60	5.00

* Boiling point.

To test the effect of oxygen the same procedure was followed as in the proceeding experiment except that oxygen was added. The data are given in table 3.

The results obtained from this run show that a maximum dissolving power of milk is obtained even in the presence of oxygen. The results approximate those obtained when pasteurized milk was used but are higher than when raw milk was used. Oxygen therefore cannot account wholly for the copper solubility of milk because raw milk should contain more oxygen than pasteurized milk and therefore should dissolve more copper, but the converse is true. The fact that a maximum is obtained in the solubility curve, at about the same temperature as is obtained with raw or pasteurized milk, shows that there is a reaction over which oxygen has no control.

CONCLUSIONS

1. The copper dissolving power of milk increases directly up to 140° to 145°F. and then decreases.
2. Either the removal of the milk gases or the addition of carbon dioxide decrease the copper dissolving power of milk.
3. The presence of oxygen increases the amount of copper which goes into solution but does not change the general type of the solubility curve as obtained in the other experiments.
4. Pasteurized milk is shown to dissolve more copper than raw milk at the same temperature.
5. It is concluded that some reaction takes place in the milk serum, when milk is heated for two hours in contact with bright copper, above the temperature of pasteurization, which decreases the copper dissolving power of milk.

AN UNAPPRECIATED BUT IMPORTANT FACTOR IN THE PASTEURIZATION OF MILK*

J. M. SHERMAN, C. N. STARK AND PAULINE STARK

Department of Dairy Industry, Cornell University, Ithaca, New York

That physiological differences exist in bacterial cells of various ages was shown by Sherman and Albus (5). They found that young cells of bacteria are more easily killed by various harmful agents, including heat, than are the old cells of the same organisms. In their paper were suggested a number of practical applications which might be made of these observed physiological differences in bacterial cells. With respect to pasteurization it was stated:

> To cite another example, in an entirely different field of bacteriology the pasteurization of milk may be mentioned. As has been shown by Ayres and Johnson (1913), the efficiency of pasteurization can not be judged, as was formerly thought, on the basis of the percentage of bacteria destroyed. It is found that in the case of milks with high bacterial counts (in which there has necessarily been an extensive growth of bacteria) it is easy to obtain an efficiency above 99 per cent, while with milks of low bacterial count (in which there has been little or no proliferation of cells) no such percentage destruction is obtained. It is of course recognized that this fact is probably due in the main to the differences in the types of bacteria predominating in these classes of milks, but it is possible that the physiological condition of the cells with respect to age also plays some part.

Although the factor of physiological youth in bacteria has not heretofore been considered by workers on pasteurization, a number of other applications have been reported by other investigators. As examples, it has been shown that the age of the bacterial cells dealt with is important in connection with the determination of thermal death rates (Robertson (3)), and (Stark

---

* **Received for publication March 18, 1929.**

and Stark (8)); in studying the germicidal action of milk (Sherman and Curran (6)); in making bacterial plate counts (Albus (1)); in the control of gassy fermentations of Swiss cheese (Rogers (4)); in studies of the rate of fermentation by bacteria (Stark and Stark (8)); and in studies of heat production by bacteria (Bayne-Jones and Rhees (2)).

EXPERIMENTAL

In another paper Stark and Stark (8) have reported the results of a number of experiments dealing with three important milk bacteria, *Streptococcus fecalis*, and two different types of ropy milk organisms. A number of strains of each of these organisms were studied and in every case it was found that at the temperature of pasteurization (62.8°C. for thirty minutes) the young cells of these organisms were destroyed more rapidly than were the mature cells. In table 1 are reproduced one typical test with each of these organisms. The young cells of *Streptococcus fecalis* were obtained by growing the culture in milk for four hours at 37°C., while old cells were procured from a culture which had been grown for twenty-four hours under the same conditions. In the case of the ropy milk organisms the cells were obtained from six- and fifty-four-hour cultures incubated at laboratory temperature. In each case the culture was trans-

TABLE 1

Influence of pasteurization on pure cultures of young and mature bacterial cells

TYPE	AGE	BACTERIAL PLATE COUNTS		RATIO BEFORE:AFTER	PER CENT SURVIVAL
		Before pasteurization	After pasteurization		
	hours				
1	24	149,500,000	3,250,000	46:1	2.17
	4	11,050,000	10,900	1,014:1	0.0986
2	54	91,000,000	45,000	2,022:1	0.049
	6	98,000	<1	>98,000:1	<0.001
3	54	21,900,000	6,750	3,244:1	0.0308
	6	485,000	<1	>485,000:1	<0.0002

ferred to sterile skimmed milk before subjecting it to the heat test.

The practical significance of the age of the bacterial cells in the pasteurization of milk is plainly evident from these data. Of particular interest are the results obtained with these two types of ropy milk organisms which reveal the fact that they may be entirely eliminated from milk if the cells are in the young and actively growing condition, while the old cells of the same organism may withstand this treatment. It has frequently been stated that the common causes of ropy milk are destroyed by efficient pasteurization, whereas it is now demonstrated that the efficiency of pasteurization for this purpose is dependent upon physiological age of the bacteria. As a matter of fact, the two types of slime producing bacteria used for these experiments were obtained from a milk plant which was experiencing difficulty with ropiness in its pasteurized product.

While there can be little doubt that such pure culture observations as have been cited are of practical importance to the dairy industry, it is desirable to have specific data on normal milk and its mixed bacterial population. It is obvious that if milk after production is kept at a low temperature so that its bacterial content does not increase, the bacteria contained therein will be old. On the other hand, it is equally obvious that if the milk is held at higher temperatures, so that the bacterial population multiplies, the cells of the microörganisms present will be relatively young. This being the case, we would expect to obtain a higher percentage efficiency in the destruction of bacteria in the case of milks in which growth has taken place. In table 2 is presented the result of a simple experiment which shows the validity of this deduction. Two samples of milk, one with a relatively low bacterial content and the other with a relatively high bacterial content, were held for twenty-four hours at 40°F. (4.4°C.). At the end of this period, each sample was divided into two portions, one portion of each being pasteurized at 145°F. (62.8°C.) for thirty minutes, while the other portion was incubated at 37°C. for four hours and then submitted to the pasteurization treatment. Bacterial counts by the plate culture method were made on all portions immediately before and after pasteurization. The plating medium used in all of these experiments was milk powder agar and the plates were incubated at 37°C. for three days.

From the data given in table 2 it is seen that the proportion of bacteria killed in the samples which were held at 37°C. is much greater than in the same samples which were pasteurized immediately after storage at low temperature. In other words, it is again shown that young bacterial cells are much more sensitive to heat than are old cells; and further, that this physiological difference in bacteria is of practical significance in the pasteurization of milk.

TABLE 2

The effect of the age of bacterial cells in a mixed milk flora on the efficiency of pasteurization

SAMPLE NUMBER	AGE	INCUBATION TEMPERATURE	BACTERIAL PLATE COUNT		RATIO BEFORE.AFTER	PER CENT SURVIVAL
			Before pasteurization	After pasteurization		
	hours	°C.				
1	24	4.4	10,100	3,250	3:1	32.17
1-A	4	37	490,000	2,400	205:1	0.489
2	24	4.4	575,000	460	1,250:1	0 08
2-A	4	37	82,000,000	28,500	2,877:1	0 0347

1-A = Sample 1 after being incubated for four hours at 37°C.
2-A = Sample 2 after being incubated for four hours at 37°C.

It is, of course, appreciated that the results thus far reported might be criticized as not being "practical," as it is a rather far cry from 37°C. to the temperatures which are more commonly used for the storage of milk supplies. It remains to be shown, therefore, that this factor of physiological youth in bacteria is an actual one under the conditions attaining in the commercial handling of milk supplies. If the principle holds, we would expect to find a greater percentage efficiency when pasteurization is applied to milk which has been held at 60°F. (15.5°C.) than when applied to fresh milk or milk which has been held at temperatures below the limits of growth of the normal milk bacteria.

In order to test this hypothesis, the following experiments were conducted: Various samples were obtained which represented milks soon after production and which had been cooled so that bacterial growth was prevented. All of these samples were of milks produced under reasonably sanitary conditions. One of the samples (as may be noted from the high bacterial count) was produced under conditions not so satisfactory and with unsterile utensils. Bacterial plate counts were made of the fresh samples. Each sample was then divided into two portions. One of these portions was pasteurized immediately while the other was placed at the holding temperature used and held for twenty-four hours. At the end of this holding period, the storage portion was pasteurized. In every case plate counts were made immedi-

TABLE 3

Bacterial efficiency of pasteurization applied to milk when fresh and held at different temperatures

SAMPLE NUMBER	AGE	INCUBATION TEMPERATURE	BACTERIAL PLATE COUNT		RATIO BEFORE:AFTER	PER CENT SURVIVAL
			Before pasteurization	After pasteurization		
	hours	*°C.*				
1	0		3,700	250	15:1	6.75
	24	4 4	4,850	50	97:1	1.03
	24	15 5	149,000	135	1,103:1	0.09
2	0		12,200	170	71:1	1.39
	24	4 4	6,200	725	9:1	11.7
	24	15 5	4,600,000	660	6,969:1	0.014
3	0		17,300	140	123:1	0.809
	24	4 4	5,850	60	97:1	1 02
	24	15 5	335,000	75	4,466:1	0.022
4	0		885,000	1,100	804:1	0.124
	24	4.4	575,000	460	1,250:1	0.08
	24	15 5	3,950,000	620	6,370:1	0.015
5	0		5,900	7	842:1	0.118
	24	4.4	6,200	4	1,550:1	0 064
	24	15.5	26,000	7	3,714:1	0.027

ately before and immediately after pasteurization. The results of five experiments in which storage temperatures of 40°F. (4.4°C.) and 60°F. (15.5°C.) were used are given in table 3.

A study of these data reveals clearly that the bacterial population of milk becomes more sensitive to pasteurization when held at 60°F. (15.5°C.). It will also be noted that a considerable increase in numbers of bacteria took place at this temperature. In other words, the bacterial population was made up of relatively young cells and was, therefore, less resistant to heat. On the other hand, in the samples held at 40°F. (4.4°C.) the plate counts indicate that no appreciable growth took place and in these cases the bacterial cells did not become more sensitive to heat.

DISCUSSION

From the observations recorded in this paper, the general conclusions seem justified that a higher percentage destruction of bacteria by pasteurization will occur in milks which have been held at temperatures which allow the multiplication of the bacteria contained therein, than in milks of the same original flora which have been stored at temperatures sufficiently low to inhibit bacterial growth. It is further shown that this difference in resistance to heat may be explained on the basis of the difference in ages of the bacterial cells involved. In this connection it should be recorded that the results reported in the foregoing tables have been verified by a number of other experiments which have not been tabulated in this paper.

The practical results obtained when milks are held at 40°F. (4.4°C.) and 60°F. (15.5°C.) are in perfect accord with what would be expected on the basis of present knowledge of bacterial physiology. The point might well be raised as to what behavior should be expected if milk has been stored at 50°F. (10°C.). From the works of numerous investigators, it is well known that milks frequently show little or no increase in bacterial numbers when held at this temperature for 24 hours, whereas, in other samples a significant increase in bacteria takes place. In other words, it would be expected that the relative destruction of bacteria by pasteurization in milks held at 50°F. (10°C.) would show considerable irregularity. For these reasons we have not emphasized our results obtained upon milk held at this temperature. The obvious expectation would be to find in milks stored at 50°F. (10°C.) a variation in behavior, in some cases simulating the results obtained at 40°F. (4.4°C.), and at other times obtaining results comparable to those secured at 60°F. (15.5°C.), depending upon whether bacterial growth had occurred. This, in fact, was found to be the case. Another irregularity in milk held at 50°F. (10°C.) has been noted: In one case a sample of milk held at this temperature showed no measurable increase in plate count during the twenty-four-hour period, but the results after pasteurization indicated a marked increase in the susceptibility of the bacteria to pasteurization. This particular irregularity is to be expected from what is known of bacterial physiology. It is well known that old bacterial cells pass through a dormant, or "lag," period before multiplication begins. As has been shown by Sherman and Albus (7), during this period of dormancy, the old cells, just prior to the initiation of growth, assume the same physiological characteristics as young cells. It is, therefore, logical to expect that when milk is kept at a temperature of 50°F. (10°C.), where growth proceeds slowly, that we should occasionally experience a marked increase in susceptibility of the bacteria to heat without an increase in the plate count.

Other points of practical interest should be mentioned. It is again made clear by this work that the integrity of the pasteurization process cannot be judged on the basis of the percentage destruction of bacteria. Aside from the reasons here emphasized, there are good and sufficient reasons for discarding that view and it has been, in fact, long since discredited. Those interested in milk control problems are at the present time emphasizing bacterial limits on the raw milk previous to pasteurization. Our results show the soundness of such standards, since it is entirely possible to obtain pasteurized milks of similar bacterial content when the raw milks from which they were derived varied greatly in germ content, depending upon the temperatures of holding previous to pasteurization.

With respect to the qualitative efficiency of pasteurization, the physiological age of the bacterial cells is of especial significance and is worthy of careful consideration by those interested in the practical aspects of pasteurization. It is shown, for example, that two different types of ropy milk organisms may be eliminated from milk by pasteurization when young cells are involved, while old cells of the same organisms may withstand the same heat treatment. It is perhaps pertinent to suggest that some consideration should be given to the relative thermal death rates of young and old cells of milk-borne pathogenic bacteria.

It hardly seems necessary to repeat that it is recognized that differences in the physiological ages of the bacterial cells is only one factor in the variations observed in the efficiency of pasteurization when applied to milks of varying quality and bacterial populations. Obviously, the bacterial flora of a sample of milk will not be the same with respect to relative numbers of different types present after extensive growth has occurred. Nevertheless, it is believed that the data presented establish the practical importance of the age of bacterial cells in the pasteurization of milk.

SUMMARY

The age of bacterial cells influences greatly the susceptibility of the organisms to heat, young cells being more easily killed than are older ones.

Milks which have been kept at temperatures at which bacterial growth takes place (e.g., 15.5°C.) show a greater percentage destruction of bacteria than occurs in similar milks when fresh or when held at temperatures (e.g., 4.4°C.) too low to allow multiplication of the bacteria involved.

It has been established that some important milk bacteria (e.g., "ropy milk" types) may be entirely eliminated from milk by pasteurization when the cells are in a young and growing condition, while the old cells of the same organisms are able to withstand the process.

A number of points of practical significance in connection with pasteurization are discussed.

REFERENCES

(1) Albus, William R. 1928 Some factors that may affect the plate count of milk. Jour. Bacteriol., xv, 25.

(2) Bayne-Jones, S., and Rhees, Henrietta S. 1929 Bacterial calorimetry. Jour. Bacteriol., xvi, 3.

(3) Robertson, A. H. 1928 Influence of age of culture on the heat resistance of non-spore-forming bacteria. Jour. Bacteriol, xv, 27.

(4) Rogers, L. A. 1928 Cheese investigations. *In* Report of the Chief of the Bureau of Dairy Industry, U. S. Dept. Agr., 1927–1928, 5.

(5) Sherman, J. M., and Albus, W. R. 1923 Physiological youth in bacteria. Jour. Bacteriol, viii, 127–139.

(6) Sherman, James M., and Curran, Harold R. 1924 The germicidal action of milk. Proc. Soc. Exper. Biol. and Med., xxii, 15–17.

(7) Sherman, James M., and Albus, William R. 1924 The function of lag in bacterial cultures. Jour. Bacteriol., ix, 303–305.

(8) Stark, C. N., and Stark, Pauline 1929 Physiological differences between young and old bacterial cells. Jour. Bacteriol., xvi, 2–3.

FURTHER STUDIES OF THE RÔLE OF VITAMIN C IN THE NUTRITION OF CALVES*

L. M. THURSTON, L. S. PALMER AND C. H. ECKLES

University of Minnesota, St. Paul, Minnesota

A previous publication (1) has reported experiments showing that calves do not require vitamin C in their feed in quantities that can be measured by the present method of testing food materials for their antiscorbutic potency by feeding them to guinea pigs. Further results have been obtained which have no effect on the practical phase of this problem, but which have extended somewhat our knowledge of vitamin C in relation to the bovine. This paper is concerned with a study of the presence of vitamin C in the livers of two of the calves and in the milk of a heifer grown from birth on the vitamin C-deficient ration and with an attempt to locate the source of vitamin C supply to calves receiving rations deficient in this vitamin.

VITAMIN C TESTS OF LIVERS

After one year of experimental feeding as previously described (1) the livers of control calf E-44, and experimental calf E-45 were removed at autopsy and immediately frozen in a sharp freezer. The antiscorbutic value of these organs was then tested by experimental feeding of guinea pigs.

Ten guinea pigs of approximately 3000 grams weight were used in this experiment. To insure the health of the animals, especially as regards their freedom from scurvy, they were fed a diet of green grass and grain for a period of 6 days before experimental feeding was begun. The excellent condition of these animals was indicated by their glossy, smooth hair, bodies

---

* Received for publication April 25, 1929. These data are taken from a thesis presented to the Graduate Faculty of the University of Minnesota by L. M. Thurston in partial fulfillment of the requirements for the degree of Ph.D. Published by permission of the Director as Paper No. 860 Journal Series, Minnesota Experiment Station.

that were plump and vigorous, and by their gain in weight during this preliminary feeding.

In this experiment a basal ration of grain mixture and timothy and wild hay was furnished. No record of the consumption of this food was kept, because it has been found (2) (3) that inanition does not produce scurvy. The animals were observed to eat the basal diet well.

The preparation of the liver for feeding consisted of chipping pieces from the frozen organ, weighing the required quantity into a porcelain dish, and adding 8 or 10 cc. of water. No attempt was made to macerate the tissue. This preparation was allowed to stand at room temperature for approximately one-half hour, after which the tissue was removed and the water extract fed. This method of preparation was resorted to because a previous trial, in which the feeding of macerated liver was attempted, gave results which were unreliable and inconclusive. The water extract of liver was force-fed by means of a syringe. It was surprising to find that, after becoming accustomed to this food, several of the animals developed a liking for it and aided in its administration. No such observation was made when the tissue itself was fed.

Both the preventive and the curative methods of study were used in this work. Four of the 10 guinea pigs were fed the extract of 10 grams of liver throughout the entire period of experimental feeding. Two of these received the extract from E-44, while the other two received that from E-45. Four other guinea pigs were fed the basal ration until the development of scurvy, after which the curative powers of extracts of the two livers were tried. The two remaining animals were used to test the antiscorbutic value of concentrated extracts of the two livers.

For the preparation of the concentrated extracts 1000 grams of liver were allowed to stand for three hours in about 3 liters of distilled water acidified with hydrochloric acid. The tissue was then removed as completely as possible by straining through cheese cloth, after which the volume of the extract was reduced to about 500 cc. by evaporation under a 25-inch vacuum. By this means the temperature of the liquid never exceeded 65°C.

A thick brown liquid resulted, which was stored in a bottle, and covered with paraffin oil to avoid oxidation of the vitamin.

Two guinea pigs received 10 cc. of this preparation daily in addition to the basal ration. One of the animals fed this concentrated material died soon after experimental feeding was begun. Autopsy failed to reveal the cause of death but a toxicity of the preparation was blamed. No symptoms of scurvy were evident in this animal.

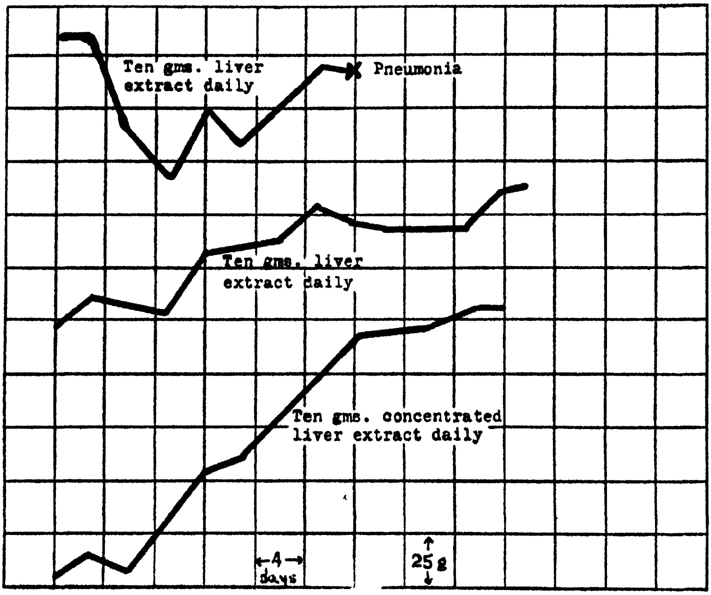


CHART 1. ANTISCORBUTIC VALUE OF LIVER OF E-44

Weight curves of guinea pigs fed extract of liver from control calf E-44. Ration, grain mixture, timothy and prairie hay ad libitum, and extract of liver. One guinea pig died of pneumonia after making gains for 15 days, but the other two remained healthy. The third guinea pig, fed a concentrated extract of the liver, made excellent gains, and showed every sign of excellent health at the close of the experiment. The antiscorbutic value of the liver here demonstrated was expected, because, previous to this test E-44 had been receiving 325 cc. of tomatoes daily for a year.

The results of the liver feeding experiment revealed considerable antiscorbutic value in the organs of both the experimental and control calves. Except for guinea pig 3, which died from abscessed lungs, the animals fed the unconcentrated extract of frozen liver lived and gained in weight throughout the 41 days of experimental feeding. Cures of scurvy were difficult to bring about because, in most cases, the disease was too far advanced before it was noticeable. However, one cure was effected. The one animal fed the concentrated product from the liver of the control calf, showed the best development of any of the experimental animals. At the close of the experiment it was the most vigorous of the entire experimental group.

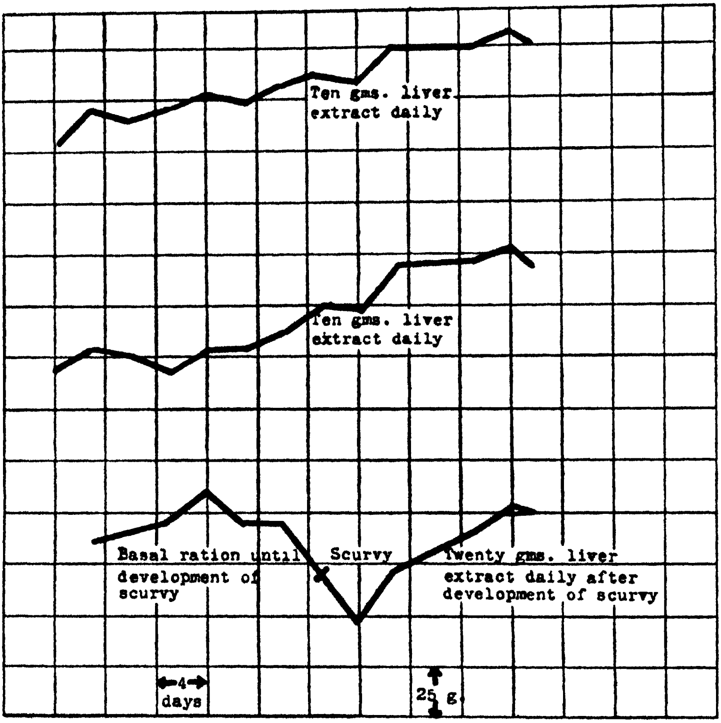


Chart 2. Antiscorbutic Value of Liver of E-45

Weight curves of guinea pigs fed extract of the liver of experimental calf E-45. Ration, grain mixture, timothy and prairie hay ad libitum, extract of liver. Two of the guinea pigs showed good gains throughout the experiment and were healthy at the close. The third guinea pig was allowed to develop scurvy on the basal diet after which a cure was effected by the daily administration of the water extract from 20 grams of liver. E-45 had been fed the vitamin C-deficient ration for one year previous to this test.

The weight curves of those animals which lived long enough to give definite results are shown in charts 1 and 2.

Antiscorbutic value of milk of E-39. When E-39 calved she had been fed since birth on the scorbutic ration. For three months previous to her birth her dam had also received the scorbutic ration. Her calf was normal in every respect. After parturition she was maintained on the experimental ration and a study of the antiscorbutic value of her milk was made. Twenty guinea pigs, weighing approximately 250 grams, were fed, ad libitum, a basal ration of the mixed timothy and wild hay, and the grain mixture used in the calf-feeding experiment. Ten of the guinea pigs received a supplement of 30 cc. of the milk of E-39 daily, and the other ten received 60 cc. Other conditions were maintained as in the other guinea pig experiments except that the animals were weighed every third day in place of every second day.

All the animals consumed the milk well. Considerable growth occurred in both groups, but those receiving 30 cc. succumbed to scurvy in from 29 to 47 days, while those receiving 60 cc. lived much longer. The experimental feeding was discontinued after 83 days. Six of the animals had died of scurvy while four still remained alive. These results make it evident that when the bovine receives no antiscorbutic in its food from the time of birth to parturition, it still secretes appreciable quantities of the factor in its milk.

Antiscorbutic value of hay concentrate. The discovery of vitamin C in the liver of a calf fed for one year on a ration deficient or lacking in this factor leaves the question of the calf's requirement for vitamin C unsettled. The possibility that the bovine requires the factor, and that the ration supplied it in quantities too small to be measured by tests with guinea pigs immediately suggested itself. Grain has so often been found to be scorbutic that any study of it as a source of vitamin C seemed needless. Less work had been done with the dry hays, and an experiment was therefore planned to attempt to detect minute quantities of the antiscorbutic vitamin in the mixed timothy and wild hay from the lot fed the calves in experiment 2.

The futility of attempting to demonstrate antiscorbutic value in this day by direct feeding was emphasized by the previous test of the hay with guinea pigs. Concentration of the water-soluble constituents of the hay offered the most logical approach to the problem, and was the method adopted.

Thirty-six pounds of ground timothy and wild hay were placed in earthen jars and covered with distilled water, buffered by monobasic potassium phosphate to maintain an acid reaction. These suspensions were held for twenty-four hours at a temperature of approximately 4°C. The resulting brown extract was then removed and concentrated in vacuo to about one-fifth its original volume. About 2½ gallons of thick brown liquid resulted. This was treated with 2 gallons of absolute alcohol which produced a voluminous precipitate. After standing over night at 4°C., it was filtered, slightly acidified with acetic acid and evaporated under vacuum to a volume of about 1 liter. Following the procedure of Zilva (4), basic lead acetate was added until no more precipitate formed. The solution was then distinctly alkaline to litmus. According to the work of Zilva (4), as well as that of Bezssonov (5), this precipitate should contain vitamin C if any were present. The precipitate was repeatedly washed by suspending it in water containing basic lead acetate, allowing to settle and then decanting. This washing was found necessary because a previous trial had shown that some poisonous principle was held by the basic lead acetate precipitate, and that its removal could be effected by continued washing. After about six washings, the precipitate was suspended in a small quantity of acidified water and deleaded. A clear colorless liquid resulted which was made up to 500 cc. with distilled water and stored in glass at ice box temperature. Paraffin oil was placed over the surface of the preparation to prevent oxidation of any vitamin C present.

Five guinea pigs were fed a basal ration of timothy and wild hay, and the experimental grain mixture, with additions of 5 cc. of the concentrated preparation. Scurvy developed very quickly. It was assumed, therefore, that no trace of vitamin C was supplied the experimental calves in their hay.

Weight curves of the guinea pigs used in this experiment are shown in chart 3.

Antiscorbutic value of feces. The results of the foregoing experiments had practically eliminated the ration as a direct source of vitamin C to the experimental calves. It therefore became reasonably certain that the bovine is capable of producing the antiscorbutic vitamin within its body. The possibility that the vitamin is produced at some point in the digestive tract was at once apparent. Fortunately, a test of the antiscorbutic value of the feces of calves fed the vitamin C-deficient ration had been made during the first experiment with calves.

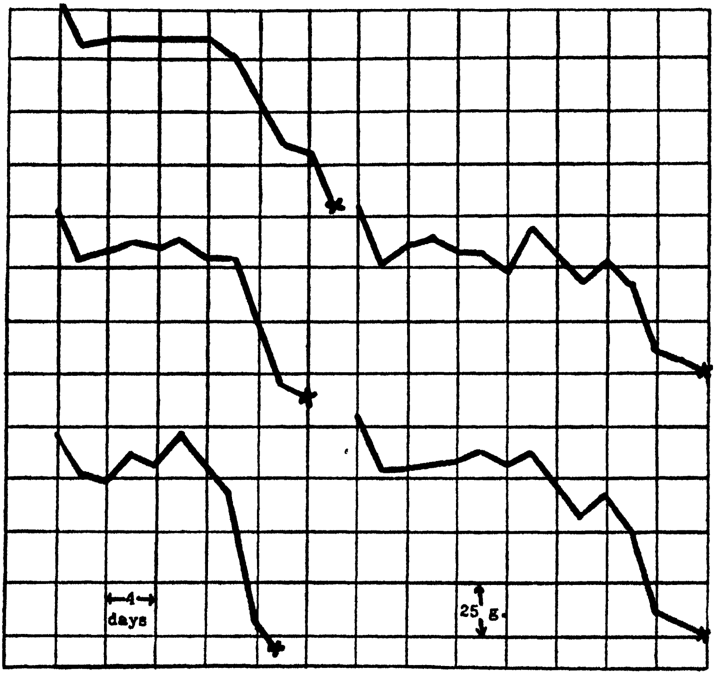


CHART 3. ANTISCORBUTIC VALUE OF CONCENTRATED EXTRACT OF TIMOTHY HAY

Weight curves of guinea pigs fed concentrated extract of timothy hay in addition to the basal diet. Ration, grain mixture, timothy and prairie hay ad libitum, 5 cc. concentrated extract. Extract equivalent to 0.36 pound of hay was fed daily but no protective action against scurvy was evident.

Nine guinea pigs, weighing approximately 300 grams, were used in this trial. They were fed a basal ration of grain and water. Feces were collected daily and fed fresh. As expected, the guinea pigs refused to eat the feces when fed separately, and it became necessary to mix them with the grain. A mixture composed of equal parts by weight of grain and fresh feces was eaten with reluctance by the animals, but proved of no antiscorbutic value. The animals died of scurvy in about 30 days. The weight curves were characteristic of those where scurvy develops quickly.

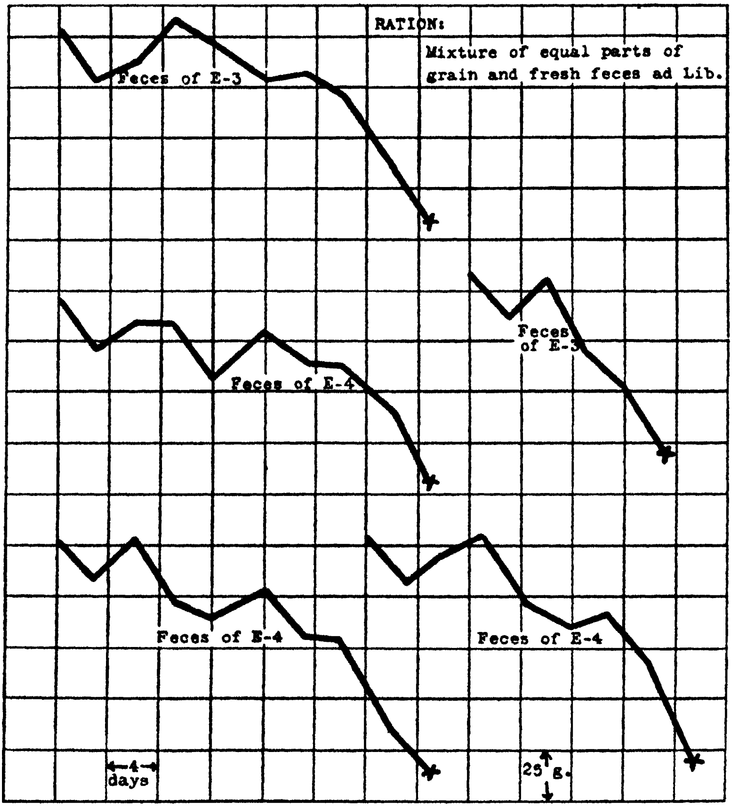


CHART 4. ANTISCORBUTIC VALUE OF FECES FROM EXPERIMENTAL CALVES

Weight curves of guinea pigs fed a mixture of equal parts of the grain mixture fed the experimental calves and fresh feces either from E-3 or E-4, both of which were fed the vitamin C-deficient diet. Vitamin C could not be demonstrated in the feces, for all the guinea pigs died of scurvy.

Table 1 shows the food consumption record for the animals used in this experiment. The weight curves are shown in chart 4.

Antiscorbutic value of stomach contents of calves fed the vitamin C deficient ration. The knowledge that vitamin C is produced in grain soaked in water for a few hours (6) led to the belief that grain in the stomachs of cattle might also be subject to this process. In studying this question an animal receiving the experimental diet of grain and dry hay was slaughtered and her stomach contents preserved by freezing. A test of these materials with guinea pigs proved unsuccessful, however, because all but one of the animals died from impaction of the stomach before scurvy could be produced. One guinea pig, fed the contents of the rumen, developed scurvy after 18 days of experimental feeding.

TABLE 1

NUMBER OF GUINEA PIG	DAYS ON EXPERIMENT	CALF STUDIED	DAILY FEED CONSUMPTION		CONDITION AT AUTOPSY
			Grain and feces	Feces (calculated)	
			grams	*grams*	
1	30	E-3	12.6	9.3	Scurvy
2	30	E-3	20 0	10 0	Scurvy
3	31	E-4	19 5	9 8	Scurvy
4	16	E-3	5 9	3 9	Pneumonia
5	27	E-4	29 5	14 8	Scurvy

Guinea-pigs weighing approximately 300 grams were used in this test. The feces were collected fresh each day and mixed with the grain in equal portions. The consumption of feces is therefore calculated on the basis that half of the feed consumed consisted of them.

This result, although inconclusive, indicates that vitamin C is not produced within the rumen.

DISCUSSION

The results of these and our previous experiments indicate conclusively that the bovine does not require vitamin C in quantities that can be detected by tests with guinea pigs. Four calves grew at a normal rate or better for a period of one year, on a ration which produced scurvy in guinea pigs in 20 to 30 days. Even when the possibility of vitamin C storage was minimized by maintaining the pregnant cow on a ration lacking this factor, the feeding of the resulting calf on a similar ration from the time of birth until one year of age failed to cause any perceptible ill-effects. Perfectly normal gestation and parturition occurred in a heifer fed from birth on a vitamin C-deficient ration. From the standpoint of the practical feeder it can be said that the bovine has no dietary requirement for vitamin C.

It cannot be said, however, that the bovine does not need vitamin C, for, even though fed for over a year on the scorbutic diet, E-39 produced milk which was normal, or nearly so, with respect to its content of vitamin C, and the factor was also found in the liver of one of the calves after a year of experimental feeding.

The results obtained in these experiments indicate that the animals did not obtain even minute quantities of vitamin C from their feed, and its synthesis within their bodies is indicated. How and where this synthesis takes place was not determined. The results from tests of the antiscorbutic value of feces and stomach contents of calves fed the scorbutic ration were all negative, and although somewhat inconclusive, due to insufficient data, all indicate that this synthesis occurs elsewhere than in the digestive tract.

The discovery of the mechanism by which the antiscorbutic vitamin is produced in the body of the calf would be of considerable technical value, because it should then be possible to determine whether or not vitamin C is required by the bovine. The similar problem presented in the rat and chicken then might also be explained. Based on the discoveries made in these experiments, the production of this vitamin by some body organ is indicated, and the enlargement of the adrenal glands in guinea pig scurvy suggests that it may be intimately concerned with this factor. Perhaps the adrenals of the bovine, rat and chicken are capable of synthesizing vitamin C, while failing of this function in man, the guinea pig and monkey.

CONCLUSIONS

1. Vitamin C can be demonstrated in the livers of calves fed for one year on a ration capable of producing scurvy in guinea pigs within 30 days.
2. Heifers fed from birth on a scorbutic diet secrete appreciable quantities of vitamin C in their milk.
3. The absence of vitamin C from the diet apparently does not interfere with reproduction in cattle.
4. Vitamin C is probably synthesized within the body of the bovine. Evidence is supplied to indicate that the digestive tract is not concerned in this synthesis.

REFERENCES

(1) Thurston, L. M., Eckles, C. H., and Palmer, L. S.: The rôle of the antiscorbutic vitamin in the nutrition of calves. Jour. Dairy Sci., 1926, ix, 37.

(2) Cohen, B., and Mendel, L. B.: Experimental scurvy of the guinea pig in relation to the diet. Jour. Biol. Chem., 1918, xxxv, 425.

(3) Anderson, W. E., and Smith, A. H.: The effect of acute scurvy on the subsequent nutrition and growth of guinea pigs. Jour. Biol. Chem., 1924, lxi, 181.

(4) Zilva, S. S.: The antiscorbutic fraction of lemon juice. Biochem. Jour., 1924, xviii, 362.

(5) Bezssonoff, N.: Quelques donnees sur la nature du principe antiscorbutique dit vitamin C. Comp. Rend. Acad. Sci. L (Paris), 1925, clxxx, 970.

(6) Honeywell, E. M., and Steenbock, H.: The synthesis of vitamin C by germination. Amer. Jour. Physiol., 1924, lxx, 321.

THE SOLUBILITY OF CALCIUM PHOSPHATE IN FRESH MILK*

E. O. WHITTIER

Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture

A knowledge of the calcium and phosphate equilibria in milk is fundamentally important, especially to investigators of nutritional and dairy manufacturing problems. No satisfactory method of determining calcium ion concentration in milk has been found and, largely for this reason, the state of the phosphate phosphorus has not been definitely determined.

Recent work by Holt, La Mer and Chown (1), by Hastings, Murray and Sendroy (2), by Sendroy and Hastings (3), and by Shear and Kramer (4) has done much toward clarifying the physico-chemical phase of the deposition of calcium salts in bone. The present article attempts to transfer to milk equilibria the results of these investigations on the behavior of the phosphate-calcium system, and to calculate therefrom the calcium ion concentration and the calcium-phosphate ion product of milk. It is realized that some of the assumptions made are open to criticism and it is hoped that both the assumptions and the calculations will be examined critically, but with constructive purpose.

A structure built on any assumption regarding the degree to which the calcium of milk is ionized is rather insecure on account of the presence in milk of considerable quantities of citrate and phosphate and on account of the comparatively large quantity of calcium itself. The assumption of complete ionization of calcium in blood serum, on the other hand, seems justified for mathematical purposes and has been made in the calculations of the investigators cited above. That, however, at least a part of the calcium of blood serum exists as a slightly, or entirely, un-ionized but diffusible compound has been indicated by work of Green-

---

* Received for publication January 14, 1929.

wald (5), Klinke (6), Sendroy and Hastings (3), and Shelling and Maslow (7). Shear and Kramer (4, Paper V) have shown by conductivity titration of calcium chloride solution "direct evidence for the binding of calcium ions by sodium citrate in some kind of soluble complex." This situation may be of consequence in the case of milk.

That the ion product $[Ca^{++}] \times [HPO_4^{--}]$ is the criterion for precipitation of calcium phosphate was the conclusion of Shear and Kramer (4, Paper III) from calcification experiments. From experimental data they derived the following expression for the negative logarithm of the corresponding solubility product: $pK_{s.p.}$ $CaHPO_4 = 6.5 - 2.6\sqrt{\mu}$ in which μ is the ionic strength, the sum of one-half the products of the molarity of each species of ion and the square of its valence. They claimed that the existence of a compound $Ca_3(PO_4)_2$ has never been demonstrated.

They found further that in the experiments of Holt, La Mer and Chown (1) and of Domontovitsch and Sarubina (8) "in which final equilibrium was attained by shaking precipitates of calcium phosphate with their supernatant solutions" a "constant" was obtained "when the analytically determined calcium concentration of the solution, expressed in mols per liter, was divided by the hydrogen ion concentration." This "constant" was found to increase with increasing ionic strength of the solution. The expression for this relationship was derived as:

$$\log \frac{[Ca^{++}]}{[H^+]} = 2.4 + 2.2\sqrt{\mu}$$

From the equation given above and from analytical data on milk serum has been calculated the calcium ion concentration, $[Ca^{++}]$, of milk at the hydrogen ion concentration of pH 6.6. In the first calculation of μ, $[Ca^{++}]$ was disregarded. The value for $[Ca^{++}]$ obtained in the calculation was then inserted in the data sheet (figures in parenthesis) and the whole calculation repeated in order that the final value might be consistent with that used for calculation of ionic strength. The second value checked with the first within the limits of proportional accuracy implied by the equation. The analytical data used were those of Van Slyke and Bosworth (9) on milk serum. The total citric acid was reduced by an amount equivalent to the serum calcium on the assumption that this amount of citric acid was removed from the ionic condition as a calcium citrate complex in accordance with the ideas quoted above. The remaining "residual" citric acid was made the basis for the calculation of the contribution of the citric acid to the ionic strength. The equations derived by

TABLE 1

The ionic strength of normal fresh milk serum. pH = 6.6

	DISSOCIATION CONSTANT	CONCENTRATION		IONIC STRENGTH, ½ (MOLARITY) (VALENCE)²
		grams per liter	*mols per liter*	
K^+		1.24	0.0318	0.0159
Na^+		0.57	0.0249	0.0125
Mg^{++}		0.08	0.0033	0.0066
Ca (milk)		1.27	0.0318	
Ca (serum)		0.465	0.0116	
Ca^{++}			(0.00028)	(0.0006)
H^+			0.0_43	
OH^-			0.0_64	
Cl^-		0.81	0.0229	0.0115
P_2O_5		1.42	0.200 [P]	
$H_2PO_4^-$	1×10^{-2}		0.0143	0.0072
$HPO_4^=$	1×10^{-7}		0.0057	0.0114
$PO_4^{\equiv}$	4×10^{-13}		0.0_89	
H_3Cit (total)		2.37	0.0123	
H_3Cit (residual)			0.0047	
H_2Cit^-	1×10^{-3}		0.0_52	
$HCit^=$	4×10^{-5}		0.0004	0.0008
$Cit^{\equiv}$	3×10^{-6}		0.0043	0.0194
				$\Sigma\mu$ = 0.0853 (0.0859)

$$\sqrt{\mu} = 0.2921 \ (0.2931)$$

$$\log \frac{[Ca^{++}]}{[H^+]} = 2.4 + 2.2\sqrt{\mu} = 3.04$$

$$\frac{[Ca^{++}]}{[H^+]} = 1096. \quad [Ca^{++}] = (1096)\ (0.251 \times 10^{-6}) = 0.00028$$

$$[Ca^{++}] \times [HPO_4^=] = (0.00028)\ (0.0057) = 0.160 \times 10^{-5}$$

$$pK_{s.p.}\ CaHPO_4 = 6.5 - 2.6\sqrt{\mu} = 5.74. \quad K_{s.p.} = 0.182 \times 10^{-5}$$

Holt, La Mer and Chown (1) were used for calculating the distribution of the phosphoric and citric acids among the three ionic forms of each. The results of these calculations are shown in table 1.

The value 0.00028M for calcium ion concentration obtained by this procedure represents $\frac{0.00028}{0.0318}$, or approximately 1 per cent of the total calcium of milk. Of the diffusible calcium of milk $\frac{0.00028}{0.0116}$, or 2.5 per cent, appears by calculation to be ionized. In other words, although approximately 36 per cent of the total calcium is diffusible, apparently only one part out of each diffusible 36 parts is ionized.

An attempt was made to test experimentally the validity of these calculations. In each of two 100 cc. graduates was placed a solution containing 0.0465 gram Ca as $CaCl_2$. To the contents of one was added 0.2370 gram citric acid previously dissolved and adjusted to about pH 7.0 with sodium hydroxide solution. To each was then added a solution of disodium phosphate containing the equivalent of 0.142 gram P_2O_5. Both were adjusted to the same hydrogen ion concentration, approximately pH 6.6, and immediately made up to 100 cc. with distilled water. Precipitation took place in the flask which contained no citrate; the contents of the flask to which citrate had been added became slightly opalescent, but precipitation did not occur. These results are taken to indicate that the presence of citrate in milk prevents the precipitation of calcium phosphate, an effect probably due to the previously discussed de-ionizing action of citrate on calcium which reduces the calcium ion concentration below that requisite to the solubility product of dicalcium phosphate.

SUMMARY

A calculation has been made of the calcium ion concentration of normal fresh milk. This calculation is based on recent experimental results bearing on the mutual effects of hydrogen, calcium, phosphate and citrate ions on one another.

A calculation of the ion product $[Ca^{++}] \times [HPO_4^{=}]$ in normal fresh milk gives a value less than that of the corresponding solubility product.

Experimental evidence as to the validity of the method of calculation is offered in the form of an experiment in which the precipitation of calcium phosphate was prevented by the presence of citrate.

It is concluded that the solubility of calcium phosphate in milk is affected by the amount of citrates present and that this effect is probably specific.

REFERENCES

(1) HOLT, L. E., JR., LA MER, V. K., AND CHOWN, H. B. 1925 Studies in calcification. I. The solubility product of secondary and tertiary calcium phosphate under various conditions. Jour. Biol. Chem., lxiv, 509.

(2) HASTINGS, A. B., MURRAY, C. O., AND SENDROY, J., JR. 1927 Studies of the solubility of calcium salts. I. The solubility of calcium carbonate in salt solutions and biological fluids. Jour. Biol. Chem., lxxi, 723.

(3) SENDROY, J., JR., AND HASTINGS, A. B. 1927 Studies of the solubility of calcium salts. II. The solubility of tertiary calcium phosphate in salt solutions and biological fluids. Jour. Biol. Chem., lxxi, 783. III. The solubility of calcium carbonate and tertiary calcium phosphate under various conditions. Jour. Biol. Chem., lxxi, 797.

(4) SHEAR, M. J., AND KRAMER, B. 1928 Composition of bone. I. Analytical methods. Jour. Biol. Chem., lxxix, 105. II. Pathological calcification. Jour. Biol. Chem., lxxix, 121. III. Physicochemical mechanism. Jour. Biol. Chem., lxxix, 125. IV. Primary calcification. Jour. Biol. Chem., lxxix, 147. V. Some properties of calcium citrate. Jour. Biol. Chem., lxxix, 161.

(5) GREENWALD, I. 1926. The effect of the administration of calcium salts and of sodium phosphate upon the calcium and phosphorus metabolism of thyroparathyroidectomized dogs, with a consideration of the nature of the calcium compounds of blood and their relation to the pathogenesis of tetany. Jour. Biol. Chem., lxvii, 1.

(6) KLINKE, K. 1927 State of combination of calcium in the serum. The pathological significance of the state of combination. Klin. Wochschr., vi, 791.

(7) SHELLING, D. H., AND MASLOW, H. L. 1928 The effect of sodium citrate, acetate and lactate on the ultrafilterability of serum calcium. Jour. Biol. Chem., lxxviii, 661.

(8) DOMONTOVICH, M. K., AND SARUBINA, O. V. 1925 Determination of the solubility product of dicalcium phosphate. Biochem. Z., clxiii, 464.

(9) VAN SLYKE, L. L., AND BOSWORTH, A. W. 1915 Condition of casein and salts in milk. Jour. Biol. Chem., xx, 135.

EFFECT OF COTTONSEED MEAL AND LINSEED OIL MEAL ON THE CONSISTENCY OF FECES OF DAIRY CATTLE*

C. F. HUFFMAN AND L. A. MOORE

Michigan Agricultural Experiment Station, East Lansing, Michigan

It is the common opinion of investigators and feeders of livestock that cottonseed meal is costive and linseed oil meal is laxative when fed to dairy cattle. According to Henry and Morrison (1), cottonseed meal is constipating, and should be fed with laxative concentrates such as linseed oil meal, wheat bran, or with succulent feeds such as silage or root crops. McCandlish (2) stated that cottonseed meal has a constipating effect and should be fed with laxative feeds such as linseed oil meal and wheat bran. He also stated that cottonseed meal is an excellent feed for cows on pasture. According to Eckles (3), cottonseed meal is somewhat constipating. Bull (4) stated that cottonseed meal has a costive action.

However, Ewing and Wells (5) observed that cottonseed meal in small quantities produces a normal feces, but, when it was the sole ingredient of the ration, the bowel action became slightly laxative. Wright (6) noticed that cattle fed a ration of 8 pounds of cottonseed meal and 25 pounds of cottonseed hulls with a feed of hay once a week excreted soft feces. The feces were made normal by increasing the hay in the ration. Reed, Huffman and Addington (7) reported that there was but very little difference between the rate of food passage through the digestive tract when cottonseed meal and linseed meal were fed. Curtis (8) stated that cottonseed meal is laxative when fed to horses.

The purpose of this investigation was to determine the effect of feeding cottonseed meal and linseed meal on the consistency of feces of dairy cattle.

---

* Received for publication January 4, 1929.

EXPERIMENTAL

Method used

The consistency of different feces has usually been measured by observing the physical appearance. In this investigation, the following mechanical method was used. The feces samples were taken immediately after evacuation. A 6 inch evaporating dish was filled with the fresh feces and leveled off with a smooth stick. A wooden ball with graduations upon its circumference was gently placed in the center of the feces in the evaporating dish.

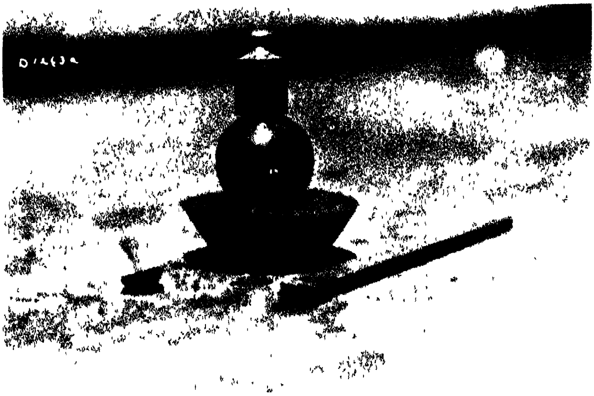


FIG. 1. SHOWING MECHANICAL METHOD USED IN DETERMINING CONSISTENCY OF FECES

Each graduation was called a degree. These graduations read from 15° to 45°. A 500-gram weight was placed upon the top of the ball which was flattened on top. The distance which the ball indented the feces was determined by the graduations. Hence, the softer the feces the further the ball sank and the greater the reading in degrees and vice versa. The determinations were made about the same time each day.

PART I

The effect on the consistency of the feces of cottonseed meal and linseed oil meal when fed with timothy hay and corn silage

The consistency of feces of six Holstein heifers which were from 24 to 30 months of age was determined. These animals

TABLE 1

Showing the effect on the consistency of the feces of cottonseed meal and linseed oil meal when fed with timothy hay and corn silage

ANIMAL NUMBER	NUMBER OF DEGREES WHICH BALL SANK IN FECES										RATION				
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	Cottonseed meal	Linseed oil meal	Corn	Silage	Timothy
Lot I															
											pounds	*pounds*	*pounds*	*pounds*	*pounds*
G 1	22	21	20	21	28	20	27	23	29	24	7.8		2	25	14
G 3	35	30	25	30	30	36	35	25	34	32	9.0		3	25	15
G 5	40	25	28	26	28	29	30	30	36	30	9.0		3.9	25	12
Daily total	97	76	73	77	86	85	92	78	99	86					
Daily average	32.3	25.3	24.3	25.7	28.3	28.3	30.7	26.0	33.0	28.7					
Lot II															
G 2	29	25	25	30	23	25	25	30	36	25		8.2		25	13
G 4	27	22	25	25	25	32	25	25	35	32		12.6	3	14	13
G 6	23	20	20	21	28	32	25	26	20	32		6.0	3	18	12
Daily total	79	67	70	76	76	89	75	81	91	89					
Daily average	26.3	22.3	23.3	25.3	25.3	29.7	25.0	27.0	30.3	29.7					

Grand total of degrees, lot I, cottonseed meal 849
Grand total of degrees, lot II, linseed oil meal 793
Difference: Cottonseed meal feces 56 degrees softer than linseed oil meal feces

are being used on a long time experiment to determine the effects on health and reproduction of heavy cottonseed meal and linseed oil meal feeding. Three animals in lot I, G 1, G 3, and G 5 have received cottonseed meal as the principal source of protein, and three animals in lot II, G 2, G 4, and G 6, have received linseed oil meal as the principal source of protein since they were 90 days of age. These observations were taken during heavy milk production. The effect of these feeds on the consistency of feces is shown in table 1. The results show very little difference in the consistency of feces of the two groups even though the cottonseed meal heifers received from 7.8 to 9.0 pounds of cottonseed meal daily and the linseed meal group received from 6.0 to 12.6 pounds of linseed oil meal daily.

PART II

The effect on the consistency of feces of cottonseed meal and linseed oil meal when fed with timothy hay and without corn silage

The results secured in part I indicated that there was practically no difference in the consistency of feces of cattle fed heavily on cottonseed meal or linseed oil meal along with silage and timothy hay. Since succulent feed, such as silage, is regarded as having a laxative effect, six mature cows were placed on an experiment to determine the effect of feeding large amounts of cottonseed meal and linseed oil meal without silage.

The cows were divided into two lots of 3 animals each. The cows in both groups received 4.5 pounds ground corn, 4.5 pounds ground oats each, and all the timothy hay which they would clean up in addition to the protein concentrate.

The animals in lot I, numbers 158, 179, and 150, received in addition, 9 pounds of cottonseed meal daily, while those in lot II, numbers 225, 226, and 217, received 9 pounds each of linseed oil meal daily. A seven day preliminary period was allowed, during which time the silage was replaced by timothy hay. The consistency of the feces excreted by the two groups was determined daily for 6 days, after which the animals in lot I were fed 9 pounds of linseed oil meal and those in lot II were fed 9 pounds of cottonseed meal daily. Two days were allowed for the change

TABLE 2

Showing the effect on the consistency of feces; of cottonseed meal and linseed oil meal when fed with timothy hay without corn silage

ANIMAL NUMBER	NUMBER OF DEGREES WHICH BALL SANK IN FECES														TIMOTHY
	First Period						Change		Second Period						
	Ration: 9 pounds cottonseed meal, 9 pounds corn and oats								Ration: 9 pounds linseed oil meal, 9 pounds corn and oats						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	
Lot I															
															pounds
155	39	40	38	36	37	30	36	39	38	32	34	41	31	40	22
150	37	35	28	37	40	31	30	31	32	36	34	30	23	31	18
179	44	34	34	38	35	33	31	28	33	36	35	38	30	38	22
Daily total	120	109	100	111	112	94	97	98	103	104	103	109	84	109	
Daily average	40 0	36.3	33 3	37 0	37 3	31 3	32 3	32 7	34 3	34 7	34 3	36 3	28 0	36 3	
Lot II															
	Ration: 9 pounds linseed oil meal, 9 pounds corn and oats						Change		Ration: 9 pounds cottonseed meal, 9 pounds corn and oats						
217	31	33	36	34	26	23	31	33	28	25	24	30	31	35	18
225	39	32	32	27	33	30	28	31	30	32	35	27	27	34	16
226	34	32	25	28	31	30	27	31	28	28	26	26	28	30	13
Daily total	104	97	93	89	90	83	86	95	86	85	85	83	86	99	
Daily average	34.7	32 3	31 0	29.7	30 0	27.7	28.7	31.7	28 7	28 3	28 3	27.7	28 7	33.0	

Sum total of degrees, lot I, 1st period, cottonseed meal... 646
Sum total of degrees, lot II, 2nd period, cottonseed meal... 524
Grand total of degrees for cottonseed meal..............1170

Sum total of degrees, lot I, 2nd period, linseed oil meal. 612
Sum total of degrees, lot II, 1st period, linseed oil meal. 556
Grand total of degrees for linseed oil meal............1168

Difference: Cottonseed meal feces 2 degrees softer than linseed oil meal feces

from cottonseed meal to linseed oil meal and vice versa. The consistency of the feces from both groups was then observed daily for 6 days.

The effect of these feeds on the consistency of the feces is shown in table 2. It is apparent that there is but little difference between the consistency of feces of animals fed cottonseed meal and linseed oil meal. It is also of interest to note in tables 2 and 3 that the change from cottonseed meal to linseed oil meal and vice versa failed to produce a disturbance in the digestive tract. These animals were not accustomed to having cottonseed meal in their ration.

PART III

The effect on the consistency of feces of only two pounds of cottonseed meal and linseed oil meal when fed with timothy hay as a roughage

The results obtained in parts I and II indicated that the heavy feeding of cottonseed meal did not affect the consistency of feces any more than the heavy feeding of linseed oil meal. However, there was a possibility that the heavy feeding of cottonseed meal caused an irritation in the digestive tract which resulted in a soft feces. In order to determine the effect on the consistency of feces of feeding a small amount of cottonseed meal and linseed oil meal, 6 animals were placed on experiment.

The cattle in lot I, numbers 202, 194, and 1 B were fed 2 pounds of cottonseed meal, 3 pounds of corn and oats daily, with timothy hay for roughage. The cattle in lot II, numbers 153, 173, and 2B, received 2 pounds of linseed oil meal in addition to 3 pounds of corn and oats daily with timothy hay.

A seven day preliminary feeding period was allowed, after which, the consistency of feces was determined daily for eight days. Animals in lot I were then fed 2 pounds of linseed oil meal in place of cottonseed meal, and those in lot II were fed 2 pounds of cottonseed meal in place of 2 pounds of linseed oil meal. These changes were made in one day. Observations were made daily on the feces for eight days thereafter. The results are shown in table 3.

TABLE 3

Showing the effect on the consistency of feces of only two pounds of cottonseed meal and linseed oil meal when fed with timothy hay as a roughage

ANIMAL NUMBER	NUMBER OF DEGREES WHICH BALL SANK IN FECES																	TIMOTHY
	First period								Change	Second period								
	Ration: 2 pounds cottonseed meal, 4 pounds corn and oats									Ration: 2 pounds linseed oil meal, 4 pounds corn and oats								
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	
Lot I																		
																		pounds
1 B	35	25	38	36	33	20	20	25	35	25	25	21	21	20	21	28	25	14
202	20	15	20	17	16	15	15	20	15	15	20	20	20	20	25	20	25	20
194	28	25	26	33	30	35	22	25	27	20	21	25	28	25	24	30	30	20
Daily total	83	65	84	86	79	70	57	70	77	60	66	66	69	65	70	78	80	
Daily averages	27.7	21.7	28.0	28.7	26.3	23.3	19.0	23.3	25.7	20.0	22.0	22.0	23.0	21.7	23.3	26.0	26.7	
Lot II																		
	Ration: 2 pounds linseed oil meal, 4 pounds corn and oats								Change	Ration: 2 pounds cottonseed meal, 4 pounds corn and oats								
2 B	36	26	27	31	30	25	26	25	27	32	25	25	25	25	27	28	25	14
153	35	38	35	35	35	33	38	35	38	43	35	38	30	30	37	35	38	22
173	31	30	36	43	30	30	28	30	30	23	21	28	30	25	33	30	35	20
Daily total	102	94	98	109	95	88	92	90	95	98	81	91	85	80	97	93	98	
Daily average	34.0	31.3	32.7	36.3	31.7	29.3	30.7	30.0	31.7	32.7	27.0	30.3	28.3	26.7	32.3	31.0	32.7	

Sum total of degrees, lot I, 1st period, cottonseed meal.. 594

Sum total of degrees, lot II, 2nd period, cottonseed meal.. 723

Grand total of degrees for cottonseed meal..........1317

Sum total of degrees, lot I, 2nd period, linseed oil meal.. 554

Sum total of degrees, lot II, 1st period, linseed oil meal.. 768

Grand total of degrees for linseed oil meal............1322

DISCUSSION

The method of judging the consistency of feces by physical appearance depends largely on the judgment of the investigator. The use of a wooden ball with graduations to measure the depth to which the ball sinks into different feces when a 500-gram weight is placed on it is a superior method of measuring the consistency of various feces.

Heifers which were raised on cottonseed meal as the principal source of protein from three months of age have excreted, for more than two years, feces which are just as soft as those excreted by animals raised on linseed oil meal for the same period. In this investigation, the amount of cottonseed meal or linseed oil meal did not affect the consistency of the feces.

When silage was left out of the ration in part II and cottonseed meal and linseed oil meal were fed at the rate of 9 pounds per day, no difference could be shown in the consistency of the feces. A sudden change from cottonseed meal to linseed oil meal feeding, and vice versa, had no apparent effect on the consistency of the feces. Cows which were fed only 2 pounds of cottonseed meal or 2 pounds of linseed oil meal daily excreted feces of about the same consistency. Apparently, the levels at which cottonseed meal was fed in these rations had no effect on the consistency of feces. The results support the findings of Ewing (5), Wright (6), Reed, Huffman and Addington (7), and Curtis (8) who observed no costive effect from feeding cottonseed meal.

SUMMARY

The results of this investigation indicate that cottonseed meal is not a costive feed for dairy cattle.

The consistency of feces of dairy cows is not altered by changing rapidly from a heavy cottonseed meal ration to a heavy linseed oil meal ration.

There was no appreciable difference between the effects of feeding a large amount and a small amount of cottonseed meal on the consistency of feces.

REFERENCES

(1) Henry, W. A., and Morrison, F. B. 1923 Feeds and Feeding, p. 369, 18th edition unabridged. The Henry-Morrison Company, Madison, Wisconsin.

(2) McCandlish, A. C. 1922 Feeding of Dairy Cattle, p. 155. John Wiley & Sons, Inc., New York.

(3) Eckles, C. H. 1923 Dairy Cattle and Milk Production, p. 330. The MacMillan Company, New York.

(4) Bull, Sleeter 1917 Principles of Feeding Farm Animals, p. 211. The MacMillan Company, New York.

(5) Ewing, P. V. and Wells, C. A. 1915 The associative digestibility of corn silage, cottonseed, meal and starch in steer rations, Georgia Agr. Exp Sta. Bul. 115.

(6) Wright, Captain 1889 Cotton seed hulls for fattening, Arkansas Agr. Exp. Sta. Bul. 9.

(7) Reed, O. E., Huffman, C. F., and Addington, L. H. 1928 Cottonseed meal as a feed for dairly calves, Jour. Dairy Sci., xi, 488.

(8) Curtis, R. S. 1911 Feeding cottonseed meal to draft animals, North Carolina Agr. Exp. Sta. Bul. 216.

BOOK REVIEWS

Dairy Bacteriology. BERNARD W. HAMMER, Professor of Dairy Bacteriology, Iowa State College. Published by John Wiley and Sons, Inc., New York, 1928.

The importance of microörganisms in the dairy is not appreciated as much as it should be by many who are engaged in the distribution of milk and in the manufacture of the various dairy products. One reason for this lack of appreciation has been the absence of any book which presents in an adequate manner the part which the various microorganisms play in the dairy. The book concerning which this note is being written will remove all reason for such unacquaintance with the rôles of microörganisms in milk and its products, since it presents in a clear, attractive, and adequate manner the general facts concerning the relation of microörganisms to the dairy. In its 473 pages one will find presented practically every subject in the field of dairy bacteriology, and in such a manner as to be understandable, not only to the student of dairying, but to those who are actually engaged in dairy manufacturing and who may have had little acquaintance previously with the subject of dairy bacteriology.

The general subject of milk fermentation is presented, together with the characteristics of the bacteria producing the various more important changes, such as the lactic acid fermentation, the ropy milk fermentation, and the gassy fermentation. The relation of bacteria to the spoilage of milk is presented in four chapters, one on contamination, one on reducing the contamination of milk and cream, another on the growth of microörganisms in milk and cream, and the fourth on the preservation of these products, in which is presented a complete discussion of pasteurization.

Approximately one-fourth of the book is devoted to the general subject of the spread of diseases by milk and its derivatives. The subjects of most immediate importance in this connection are Malta fever and contagious abortion; udder inflammations and septic sore throat.

The bacteriology of evaporated, sweetened condensed, and powdered milk, of ice cream, and of fermented milk is presented in three chapters.

The author has done much original work on the study of the bacteriology of butter cultures and of butter. One would therefore expect the presentation of this subject to be most adequate in the 65 pages devoted thereto. The book ends with a chapter devoted to the bacteriology of cheese and another in which are described the tests for quality of milk and cream. Included in this discussion is a presentation of the limitations of the various tests commonly used in the manufacturing plant.

The book presents a very attractive appearance, both as to its exterior and interior.

The production of milk, its handling, its distribution, and its manufacture into the various products, involve an immense amount of bacteriology. The work will be done with the greatest success only as those engaged therein give conscious consideration to the bacteriology of the raw material and of the different manufactured products.

This book should find a place on the desk of everyone who is interested in the management of any phase of dairying. It is to be hoped that the demand for the book will justify frequent revision, in order that the subject may be kept abreast of the rather rapid advances in many of its phases.

E. G. Hastings.

CONTRIBUTION TO THE KNOWLEDGE OF THE TASTE OF MILK*

C. L. ROADHOUSE†

Department of Dairy Industry, University of California, Davis

AND

G. A. KOESTLER

Department of Chemistry, Swiss Dairy and Bacteriological Experiment Station, Liebefeld, Bern, Switzerland

Very little research has been reported dealing with the taste of milk at the time it is drawn from the cow, except as it is influenced by the use of certain feeds in the rations of dairy cows or by stable odors. In the scoring of milk as carried out in the United States, certain deviations from the normal taste of milk have been recognized, but complete knowledge of the causes of these variations has been lacking.

Milk, before it is delivered to the consumer, receives certain treatment, such as aeration and pasteurization, by means of which some minor defects in flavor may be more or less successfully removed, but there may be defects of taste which can not be removed by the usual methods of handling. It has been considered worth while, therefore, to study more closely the taste of raw, untreated milk and to discuss the individual variations in taste which are apparent in the milk from different cows.

PROCEDURE

The milk score card of the United States Department of Agriculture was used. All samples were heated to a temperature of 40° to 43°C. before they were tasted. Three experienced judges examined all samples of milk and when there was not complete

---

* Received for publication March 1, 1929.

† This study was conducted by the senior author while on sabbatical leave in Switzerland during 1927–1928. The authors are indebted to Mr. W. Loertscher of the Dairy Experiment Station at Liebefeld, Switzerland, for helpful collaboration in examining the flavor of the milk.

agreement, the results were averaged. In few instances was the variation greater than two-tenths of a point in the score.

From the preliminary trials it was evident that in spite of the most careful handling of the milk, including sanitation, cooling and withholding feed from the cows previous to milking, there was a difference in the taste of the milk from different cows and the individual milkings of the same cow. A careful study was made of the individual differences in taste and at the same time an effort was made to take a first step toward the analysis of the differences in taste.

The taste of milk, as this word is commonly used, is the sensation perceived when milk is taken into the mouth. The term "flavor," as used in this paper, is a combination of the sensations of taste in the mouth with those of smell produced through the medium of the inner nasal passages. The term "taste" is often used as synonymous with flavor, but it aids in precision to confine this term to those sensations which are perceived only in the mouth, and to use the term "flavor" to include also those sensations which are undetected if the milk is tasted while preventing the passage of air through the nasal passages. Odor or aroma, as applied to milk, describes the sensations observed when the milk is subjected to examination by the nasal passages alone. In tasting milk one must consider the impressions transmitted not only by the tongue, but also by the whole inner surface of the mouth. The correct taste impressions are more easily secured when the milk is taken in not too large amounts.

THE IDEAL MILK TASTE

At first thought, it appears somewhat daring to refer to an "ideal" milk taste. The taste trend of the individual consumer may vary somewhat from that of the milk-consuming public. Nevertheless, the ideas pertaining to the ideal milk taste are probably in closer agreement than are the ideas of the public concerning butter and cheese.

It is evident from the investigations which have thus far been made, that there is a type of milk which must be designated by every true connoisseur as one free from objection as far as taste is concerned. After swallowing, an absolutely agreeable sensation must be left in the mouth. The milk is without odor under the usual conditions of examination, and the taste sensation which is given through the principal milk ingredients is even and full. No part of the taste as a whole predominates in such a way that the harmony is destroyed, but rather do the individual taste characteristics of the principal ingredients blend into one another. A further important requirement is that the milk must not give the impression of being thin or watery; the presence of the water should be masked by the combined taste sensation to such a degree that a flat, watery taste is not present.

ANALYSIS OF THE MILK TASTE

In the system of milk scoring used by other workers, a sharp distinction has not been made between the natural foundation taste and the secondary taste. There is no disadvantage recognized in this system, for the purely comparative judging of the taste. Just as soon, however, as one desires to approach the "analysis of the taste," then the necessity is felt for undertaking a certain basic classification. A first step in this direction was to consider what constitutes the foundation taste of the milk. It was decided that it should include all of the taste-giving ingredients which milk normally contains.

This "primary taste" of milk must be distinguished from the "secondary taste" or "flavor," which comprises defective body and everything of a foreign nature which the milk acquires, such as feed and stable flavors.

In attempting to analyze the taste of milk it became clear that the chemical composition of milk is most concerned with the foundation, or primary taste, of average milk; that feed and stable flavors are responsible for the secondary taste, and that the physical condition of the milk, which influences the body, serves to regulate the total impression by increasing or decreasing the pleasantness of the taste. The primary taste of milk cannot be successfully judged unless the conditions leading to secondary flavors are eliminated.

THE VARIATION IN THE TASTE OF MILK FROM NORMAL COWS

When tasting samples of milk collected from 4 dairy herds in Switzerland, the writers observed considerable variation in the taste of milk from individual cows. Among 82 cows from which samples were taken, there were 8 cows which produced milk with an especially fine taste; 35, with a good taste; 11, with a slightly salty taste; 2, distinctly salty; 4, slightly rancid; 5, distinctly rancid; and 4, with a bad taste.

TABLE 1

Variations in taste and body of milk from apparently normal cows

NAME OF COW	SAMPLE NUMBER	FLAVOR SCORE	JUDGES COMMENTS	LENGTH OF TIME COW HAD BEEN MILKING
Flora	9	13.1	Slight salty, cowy	10 months, 3 days
Gritli	5	13.8	Very good taste	6 months, 24 days
Natter	1	13 8	Fine taste	9 months, 16 days
Nigeli	10	13 5	Aftertaste	4 months, 6 days
Lou	18	13.7	Slight watery	4 months, 19 days
Lusti	1	13 2	Chalky, uneven, lacks taste	10 months, 21 days
Bernina	5	13 3	Watery	1 month, 12 days
Rosette	7	10 8	Bad taste and odor, musty	16 days
Wanda	11	13 2	Slight salty	7 months, 18 days
Pupilla	14	13 0	High acid, uneven taste	3 years, 1 month, 21 days
Falk	8	13.4	Very slightly rancid	8 months, 21 days
Primula	1	13 4	Flat taste	5 months, 8 days
Rütli	17	13 7	Superb taste, thin body	
Pia	23	13 0	Acid-like taste	9 days
Spiri	4	13.1	Astringent, off taste	2 months, 1 day
Vion	9	13 0	Thin body, not pleasing	2 months, 22 days
Schägg	7	12.8	Sharp taste, salty	3 months, 9 days
Gemse	8	12.8	Thin, watery and bitter	9 months, 28 days
Blösch	6	12.8	Sharp, slightly bitter and sweet	
Türk	4	11.5	Slightly rancid odor, rancid taste	
Runde	2	12.6	Strong salty, aftertaste	
Spiess	6	12.6	Disagreeable odor and taste	
Tiger	1	0	Strong rancid odor and taste, bitter	12 months

Gamble and Kelly (1) have reported a wide variation in the taste and odor of the milk from individual normal cows receiving the same feeds, and one of us (2) has observed a similar variation in taste in milk from cows examined in California.

In table 1 are given the scores for certain cows' milk showing variation in taste. In each instance feed was not given to the cows during the five-hour period previous to milking.

The results show marked differences in the taste between certain samples of milk.

THE CHEMICAL COMPOSITION OF MILK WITH A NORMAL, PLEASING TASTE

In order to determine if the different milk samples with exceptionally pleasing primary tastes would also show certain agreements in their chemical composition, milk samples were taken from three selected cows, the milks of which were especially agreeable in taste. This was shown in the milk from the cows, Felder, Müller and Lisi. The milk from the latter cow showed a slight after-flavor, which, according to our previous definition, would be classified as secondary taste.

Table 2 gives the analytical results. It is certainly not to be considered accidental that these three milk samples with excellent primary tastes should agree in lactose content to within a few hundredths per cent. An approximate agreement in chloride content is also shown.

Without endeavoring to obtain positive proof from these three examples, it can be assumed that the agreement of the chemical analyses with the judges' impressions of taste is close. It might also be noted that no especially close agreement was found in coarsely dispersed components such as fat, protein, and some ash constituents.

These results, therefore, furnish certain confirmation of the above statement, that the coarsely dispersed components exert an insignificant influence on the primary taste of milk.

TABLE 2

Analyses of milk with an especially good and even taste

	COW		
	Müller	Felder	Lisi
Physico-chemical analyses:			
Specific gravity at 15°C.	1.02957	1.03408	1.03193
Acidity (calculated in per cent acid from Soxhlet-Henkel deg.)	0.13	0.14	0.12
Hydrogen ion concentration (pH)	6.70	6.68	6.63
Electrical conductivity (Mhos × 10^{-4})	41.79	39.37	44.09
Stalegmometer (J. Traube) S.T.:			
a. Drop count	38	37	37
b. Time (seconds)	36	36	42
Chlorine-lactose number	1.896	1.691	1.692
Chemical composition (per cent):			
Fat content (Röse-Gottl.)	3.65	3.79	4.96
Lactose hydrate	4.92	4.92	4.94
Total protein	2.639	2.591	3.599
Casein	1.85	2.14	2.83
Albumin, globulin	0.38	0.36	0.46
Citric acid	0.262	0.269	0.279
Ash	0.695	0.689	0.739
Total	12.166	12.559	14.317
Total dry matter (per cent):			
a. Calculated (Fleischman)	12.01	12.42	14.18
b. Weight analyses	11.86	12.18	13.96
In the ash:			
1 liter of milk contains (gram):			
CaO	1.582	1.579	1.911
MgO	0.035	0.121	0.173
K_2O	1.982	1.957	1.881
Na_2O	0.380	0.579	0.684
Cl	0.933	0.832	0.836
SO_3	0.192	0.235	0.303
P_2O_5	1.960	2.018	2.324
Total (ash without Fe_2O_3)	7.064	7.321	8.012
minus O corresponding to Cl	0.210	0.188	0.189
Net ash (without Fe_2O_3)	6.854	7.133	7.822

THE CHLORIDE-LACTOSE RELATION AS ONE OF THE MOST IMPORTANT BASES OF THE TASTE OF MILK

As has already been stated, those milk constituents which are naturally prominent in taste must be important in contributing to the primary taste of milk. The presence, as well as the quantity of these substances, is believed to be important.

It was expected that under otherwise similar conditions the highly dispersed substances, chlorides and lactose, with their blending properties would influence essentially the primary taste of the milk. Differentiation must be made between different conditions. A relatively high chloride-lactose number may be present in milk from normal cows advanced in lactation or it may result from a disturbance caused by udder bacteria.

In the first case one would have to consider the change in the milk as normal and the resulting change in milk taste would have to be considered as belonging to the primary taste. On the other hand, if the secretion has been disturbed by udder bacteria and the milk undergoes changes in its composition, then the chloride-lactose number may become still higher. Here one is dealing with a secondary influence on taste.

The milk from cows advanced in lactation which have a low bacterial content in their udders may show a pleasing primary taste in spite of the relatively high chloride-lactose number, since in some instances the lactose content is not essentially changed from normal. There is also no reason why an unpleasant secondary taste should be present in the milk which has undergone other slight changes in composition. Less objectionable are such cases then, since the increasing of the chloride-lactose ratio is to be considered as a result of a disturbance of secretion resulting from udder bacteria. As a rule the lactose content is materially lowered. It is to be expected that other substances, also affecting taste, will originate from the diseased udder tissues, as well as from udder bacteria present.

In this connection there is the further question as to the chemical combination of the chloride in milk. Soldner (4) and Porcher and Chevallier (5) have considered the chloride under normal conditions as combined with sodium and potassium. Van Slyke and Bosworth (6) assume that the chloride of normally secreted cow's milk is united with the calcium. In this connection it would appear that a distinction must be made between the condition of the chloride which is present in the normally secreted milk and that which is secreted from abnormal udders. Whereas, the chloride does not need to be present with large amounts of sodium ions in all milks, it can probably be assumed with certainty that the chloride ion in salty milks, under abnormal conditions, is present with a relatively large amount of sodium ion. As far as the chloride of normally secreted milk is concerned, it has not been determined in which chemical combination it is present. Equal uncertainty exists as to the degree of ionization of the chlorides in the normally secreted milk. All of these questions are significant in attempting to determine the cause of certain tastes in milk.

In numerous publications (7), reference has been made to the salty and the salty-bitter tastes of milk, but the discussion of the chemical nature of this defect in taste has not been seriously considered. Gabathuler (8), in a study of the importance of lactose in nutrition, has pointed to the decrease of the lactose content in slight diseased conditions of the milk secreting tissues, while Hammer and Baily (9) in their studies on flavor, attached certain importance to the absence of alkali chlorides in milk. In the course of our own investigations the authors found that the chloride-lactose relation could regulate the foundation taste of milk to a certain degree. Milk samples with a high chloride-lactose ratio were judged less favorably than milk samples of like origin where the chlorine-lactose number was relatively low.

A possible explanation of the phenomenon is that for physiologically definite reasons the osmotic pressure of milk must be kept within certain narrow limits. Undoubtedly, the osmotic pressure of milk is largely dependent upon such substances as lactose in molecular dispersion, and the electrolytes, such as the alkali chlorides in ionic solution. The relation of salt to lactose may be due to the regulation of the osmotic pressure, in which case the chloride is taken from the blood or lymph, while

TABLE 3

The relation of chloride and lactose to taste of milk

NAME OF COW	FLAVOR SCORE	LACTOSE PERCENTAGE	CHLORINE AS CHLORIDE	CHLORIDE-LACTOSE NUMBER	JUDGES' COMMENTS
			grams per liter		
Felder	13.4	4.98	0.78	1.50	Slight sharp taste
Lisi	13.4	4.94	0.85	1.67	Pleasing taste
Müller	13.4	4.74	0.76	1.60	Very good taste
Müller	13.4	4.91	0.88	1.73	Even composition; very slightly sharp
Fuchs	13.4	4.90	1.10	2.18	Very good taste
Felder	13.3	5.23	0.76	1.39	Pleasant sweet taste; slightly off
Felder	13.3	4.86	1.02	2.02	Good milk
Müller	13.3	4.69	0.80	1.43	Even composition; good
Spiegel	13.2	5.06	1.01	1.93	Slight salty; taste not pleasing; even composition
Boni	13.2	4.70	1.24	2.59	Slight salty; composition off
Boni	13.2	4.54	1.24	2.60	Sharp aftertaste
Alice	13.2	5.08	0.77	1.45	Thin body
Lisi	13.1	4.91	0.88	1.67	Salty taste
Lisi	13.0	4.84	0.84	1.67	Aftertaste
Pfau	13.0	4.50	1.37	2.98	Slight aftertaste
Mei	13.0	4.64	1.08	2.26	Slight aftertaste; even composition
Miggi	13.0	4.24	1.42	3.27	Slight salty; aftertaste not pleasing
Gemse	12.9	4.19	1.31	3.10	Thin; watery; slight salty; slight aftertaste; sharp
Blösch	12.9	4.59	1.10	2.33	Composition off
Burgi	12.9	4.09	1.35	3.24	Bitter; salty
Schägg	12.8	4.74	0.94	1.81	Slight bitter aftertaste
Schägg	12.8	4.49	1.27	2.76	Astringent; aftertaste
Blösch	12.8	4.87	1.20	2.40	Sharp, sweet taste
Meisi	12.8	4.28	1.52	3.48	Slight salty
Gemse	12.7	4.19	1.28	2.98	Salty; off
Miggi	12.7	4.18	1.47	3.44	Slight salty
Rundi	12.6	3.74	1.73	4.54	Strong salty; aftertaste
Spiess	12.6	4.68	1.04	2.16	Disagreeable odor and taste; aftertaste
Schägg	12.5	4.77	1.13	2.30	Sharp; bitter
Spiri	12.3	4.82	0.95	1.90	Disagreeable taste
Gemse	12.3	4.22	1.35	3.12	Salty; aftertaste
Veiel	12.2	4.16	1.66	3.92	Salty; sharp (udder trouble)
Turk	11.5	4.47	1.44	3.15	Slight rancid odor and taste
Freude	11.0	4.48	1.24	2.48	Rancid odor and taste

the lactose regulation may probably result from a restraint in the percentage of lactose elaborated. The relation between chloride and lactose and the taste scores of the different milk samples may not correspond in all cases. As previously emphasized, there are instances where a defect in taste of a secondary nature may be present in addition to a high chloride-lactose ratio. The chloride-lactose relation is only one of the factors of importance in the analytical determinations of taste in cow's milk.

The value of the chloride-lactose number is identical with the chlorine-lactose number, as described by Koestler (3) and is found by multiplying by 100 the ratio of percentage chloride to percentage lactose. The chlorides reported in grams per liter in column 4 of table 3 have been changed to percentage in making the calculation to determine the chloride-lactose number.

It is apparent that the samples of milk with the higher scores on flavor had a relatively low chloride-lactose number. In all but one instance, the salty taste was accompanied by a chloride content above 1 gram per liter.

THE TASTE OF SOLUTIONS CONTAINING LACTOSE AND SODIUM CHLORIDE

In further study of the sources of the taste in milk and the influence of lactose and sodium chloride upon the taste, solutions containing lactose and sodium chloride were prepared with distilled water. The solutions contained the maximum and minimum lactose content likely to be found in milk and the corresponding chloride content. The concentration of the solutions was made to correspond with the osmotic pressure of normal milk, considering for this study that the osmotic pressure would be caused only by these two substances.

The results indicated that in warm solutions (35°C.) the taste of the salt and sugar are blended and a more pleasing taste is given than in the cold solutions (15°C.). The sugar taste seemed more apparent in the warm solutions, while the salty taste seemed more prominent in cold solutions. In the cold solutions the sugar and salt tastes seemed to be separate and were less pleasing.

The influence of the temperature upon the taste of these solutions was found to vary according to the relative concentrations of sugar and salt in the solutions.

THE INFLUENCE OF SODIUM CHLORIDE AND CALCIUM CHLORIDE ON THE TASTE AND ODOR OF MILK

Although the question as to the combination of chloride in normal milk must remain open, it seemed to be of interest to compare the influence on the taste of milk of the addition of equivalent amounts of sodium chloride and calcium chloride. For this purpose increasing amounts of sodium chloride and calcium chloride were added to different samples and they were subjected to examination for taste and odor. The results are tabulated in table 4.

When a sufficient quantity of sodium chloride was added to the milk, a salty taste resulted. The samples to which calcium chloride had been added did not give a salty taste, but the milk produced an astringent effect in the mouth. An addition of calcium chloride equal to 0.355 gram of calcium oxide per liter of milk produced a pronounced sharp, astringent taste.

When adding sodium chloride to Felder's milk to bring the content of sodium chloride to 0.1539 per cent, a weak, salty taste was present in the milk. The salty taste produced by adding a given quantity of sodium chloride was less prominent, however, than the salty taste of secreted milk with the same sodium chloride content.

It is interesting to note that the added sodium chloride and calcium chloride in certain amounts produced an odor in the milk. These salts probably change the physico-chemical properties of the milk in such a way that the milk is not able to retain certain gases which gives the impression of odor.

RELATION OF FATS AND PROTEIN TO THE TASTE OF MILK

When beginning the study of the cause of variations in the taste of normal milk, attention was naturally given to the influence of butterfat on the taste of normal milk. Several lots of mixed milk were passed through a cream separator and the taste of the skim milk compared with that of the original whole milk. Different samples gave slightly different impressions, but in general when the whole milk had a pleasing taste, smooth body, and was even in composition, the skim milk was equally pleasing in primary taste, was not so full but was nearly as palatable as the

TABLE 4

The relation of sodium chloride and calcium chloride to milk flavor

COW	SCORE OF NORMAL MILK	MIXTURES USED	NaCl ADDED*	FLAVOR SCORE	REMARKS	$CaCl_2$ ADDED*	FLAVOR SCORE	REMARKS
			grams			*grams*		
Lisi	13.2	I	0.0349	13.3	Good taste	0.03516	13.0	More flavor but aftertaste
		II	0.0698	12.9	Salty, sharp, slightly astringent	0.07032	12.9	Not pleasing taste, astringent
		III	0.1047	12.6	Distinctly salty	0 10548	12.8	Aftertaste, off, astringent
		IV	0.1396		Objectionable odor, distinctly salty			Stable-like odor, distinctly off, astringent
Müller	13.5	I	0.0349	13.0	Slightly astringent, very slight salty	0.03516	13.2	Slight odor, slightly astringent
		II	0.0698	13.0	Slight salty	0.07032	13 1	Slightly astringent
		III	0.1047	12.7	Salty, sharp	0.10548	12.8	Distinctly astringent
		IV	0.1396	12.2	Odor, distinctly salty	0.14064	12.2	Odor, off, astringent
Felder	13.1	I	0.0349	13.2	Better than original	0.03516	13.2	Astringent
		II	0.0698	13.0	Not astringent, slight salty	0.07032	12 8	Distinctly astringent, sharper than normal
		III	0.1047	12.7	Odor, salty	1.10548	12.5	Odor, astringent, strong, sharp

* Added to 100 cc. of milk.

whole milk. This supports the previous statement in this paper that the fat is not an important constituent in milk influencing the primary taste.

THE DEGREE OF DIALYSABILITY OF THE MILK INGREDIENTS AND ITS INFLUENCE UPON THE PRIMARY TASTE OF THE MILK

It was assumed from the beginning that the different milk ingredients participate in a different degree in bringing about the primary taste of the milk. It is furthermore reasonable to assume, for this purpose, that the degree of dispersity of the milk ingredients is an important item.

TABLE 5

The influence of dialysis on the taste of milk

COW	ORIGINAL MILK	TASTE OF MILK IN COLLODION SACK	MILK USED	WATER USED
			cc.	*cc.*
Lisi	Good taste, sweet, slightly thin body, score—13.4	Almost tasteless, flat, watery	120	200
Felder	Pleasing taste, very slightly watery	Almost tasteless, flat, lacked flavor, watery taste, and body almost like water	120	120

TABLE 6

The chemical analysis of one of the milk samples subjected to dialysis

ANALYSES MADE	ORIGINAL MILK	DIALYZED RESIDUE IN COLLODION SACKS	DIALYZATE IN WATER SOLUTION
Specific weight	1.0306	1 0168	1 009018
Fat percentage	3 69	3 30	
Dry matter percentage	12 32	8 60	2 10
Milk sugar percentage	4.76	2 31	1 73
Chloride—grams per liter		0.412	0 195
Ash percentage		0.0433	0 0372
Phosphoric acid		0.110	0 022
Calcium oxide percentage		0 112	0.024

In an effort to determine the correctness of these assumptions, the milk of different cows was subjected to dialysis by using the collodion filter with a fifteen hour dialysis against distilled water held at a temperature below 15°C. Table 5 gives the results of the examination of two samples which were typical of several milks similarly treated, and table 6 includes the chemical analyses of the original milk, the dialyzed residue and the dialyzate.

In order to have an exact comparison between the taste of the original milk and that of the dialyzed residue, the sample of Lisi's milk referred to in table 5 was brought to the same water content as the dialyzed residue which had been diluted during dialysis, with water drawn through the membrane.

It was manifest that the milk which was partially freed from the easily dialyzed materials was almost tasteless. At the time of tasting, the impression given was that of tasting viscous water, whereas the original milk which had been diluted to the same water content still gave essentially the taste of the original milk.

With these simple dialyzing experiments, proof is given that along with the partial removal of the dialysable substances of the milk by far the greatest part of the primary taste is also eliminated from the milk; or, in other words, the primary taste of the milk is to the greatest extent associated with the finely dispersed, easily dialysable ingredients; whereas, fat and milk protein, both of which remain almost entirely within the dialyzed residue, are concerned, if at all, to only a very slight degree with the primary taste of the milk.

It can furthermore be determined by dialysis that certain elements of the secondary taste remain in the dialyzed residue. This is evident for the reason that these elements of taste are combined in some manner with the substances (fat and protein) which can be dialyzed only with difficulty.

DISCUSSION

Certain constituents are capable of contributing to the taste of milk. Others do not produce definite taste reactions, but they do influence the taste impression by their influence upon the physical properties of the milk. Acids, because of the large quantity of hydrogen ions present, give a more prominent taste impression than do more weakly ionized materials, such as albuminoids.

Not all of the taste producing ingredients of milk give the impression of taste to the same extent nor at the same time. The water or saliva solubility of the separate ingredients is undoubtedly concerned, since the more soluble substances can be expected to give rise to the impression of taste under similar conditions sooner than do the incompletely dispersing ones.

The degree of dispersion of the milk components influences the total taste impression. Since the fat has a relatively low degree of dispersity and shows marked absorptive qualities, it may become a carrier of those flavor producing materials which are absorbed into the milk.

The degree to which the different taste impressions can be identified during the act of tasting is not an indefinite matter in the judging of milk taste. As soon as the milk has been taken into the mouth, generally those taste sensations predominate which originate from the substances in true solution, such as lactose and salts. Lactose and certain salt ingredients take an important part in the fundamental taste of the milk.

The foundation taste of milk resulting from its natural composition, is supplemented by certain secondary tastes. These give definite impressions such as acid and bitter tastes, or they represent a mixture of a non-typical sort, such as feed or stable flavor. Such secondary flavors are probably to be considered generally as "foreign" to normal milk.

The secondary flavor may very often be of a volatile, aromatic nature, and hence show itself more or less plainly in the odor of the milk. There are also secondary flavor sensations which can be classified as "aftertaste," and which evidently become concentrated upon the mucous membranes. They contribute to the sense of taste, but do not leave a distinct impression until the greater part of the milk has been removed from the mouth.

With the uncertainty which still exists concerning the identification of the different degrees of taste, it is possible to properly designate the finer sensations of taste to only a limited degree. However, one is able to think of the taste of milk as it is influenced by its constituents. As methods for the more complete analyses of milk are perfected, it should become possible to determine, to a still greater extent, the relation of the constituents of milk to its taste.

It is recognized that many phases of this subject remain to be further investigated. It is expected that others may be stimulated to contribute to this important, but hitherto neglected, field of study. The senior author is continuing research in this subject.

SUMMARY

1. The taste of milk, as this word is commonly used, is the sensation perceived when milk is taken into the mouth. The term "flavor," as used in this paper, is a combination of the sensations of taste, perceived in the mouth, with those of smell, produced through the medium of the inner nasal passages. It has aided in precision to confine the term "taste" to those sensations which are perceived only in the mouth.
2. The primary taste of milk has been designated as the sum of all of the taste impressions coming from normal milk, and not influenced by feed or the secretion of abnormal milk.
3. The secondary taste includes that which is added to the primary taste from different sources, such as feed and products of disease. It is necessary to eliminate these secondary influences in order that primary tastes can be properly determined.
4. The chloride-lactose relation is one of the most important bases of milk taste. Milk samples with a high chloride-lactose ratio were judged less favorably than those of like origin where the chloride-lactose ratio was relatively low.
5. The primary taste of skim milk is practically equal to that of the whole milk from which it is separated.
6. By the application of dialysis, it was possible to separate the milk into two parts (dialyzate and residue) with extreme differences in taste. It was found that nearly all of the milk components producing the primary taste were present in the dialyzate, while the components remaining in the residue could be designated as free from taste.

7. By dialysis it has been further demonstrated that fat and protein substances, as well as certain difficultly dialyzable salt components, which go to make up a large percentage of the milk content, take only a subordinate part in the primary taste of the milk.

8. The dialysis of milk containing a pronounced feed taste has shown that the feed taste was found very largely in the residue, and appeared to be either not dialysable or in some way combined with milk fat or with other non-dialysable materials.

REFERENCES

(1) Gamble, J. A., and Kelly, E. 1922 The effect of silage on flavor and odor of milk. U. S. D. A. Bul. No. 1097.
(2) Roadhouse, C. L. 1925-1926 Calif. Agr. Exp. Sta. Annual Report, p. 62.
(3) Koestler, G. 1920 Mitt. Lebenam Hyg., xi, 154. Cited from Fundamentals of Dairy Science, 1928.
(4) Söldner, F. 1888 Die Salze der Milch und ihre Beziehungen zu dem Berhalten des Raseins. Die landw. Bers. Stat., xxxv, p. 351.
(5) Porcher, Ch., and Chevallier, A. 1923 La repartition des matieres salines dans le lait Leurs relations physiques et Chimiques avec les antres principes du lait. Le Lait, iii, p. 97–112; 188–200.
(6) Van Slyke, L. L., and Bosworth, A. W. 1915 Condition of casein and salts in milk. Jour. Biol. Chem., xx, p. 135.
(7) Koestler, G., Steck, W., and Radosavlevitch, M. 1921 Landw. Jahrb. der Schweiz, p. 631.
(8) Gabathuler, A. 1915 Die Bedeutung des Milchzuckes für die hygienische Beurteilung der Milch. Zeitschr. f. Fl. und Mch. Hyg., xxv, p. 97.
(9) Hammer, B. M., and Baily, D. E. 1927 Relation of chlorine content to abnormal flavor and odors of milk. Iowa Sta. Res. Bul. 41.

STUDIES ON THE NUTRITIVE VALUE OF MILK

III. THE SUPPLEMENTARY VALUE OF VARIOUS CONSTITUENTS OF SYNTHETIC BASAL RATIONS*

W. E. KRAUSS

Department of Dairying, Ohio Agricultural Experiment Station, Wooster, Ohio

It has previously been reported that rats, started as weanlings, could not be raised to maturity on an exclusive whole milk diet (1) (2). This failure was attributed to the development of nutritional anemia, due to an insufficient intake of copper and iron. Inasmuch as rats had been successfully raised on synthetic rations, the problem of discovering the component or components of such a ration that would adequately supplement milk presented itself. With this object in mind, the following experiment was undertaken.

EXPERIMENTAL PROCEDURE

Albino and piebald rats were fed a basal ration of liquid whole milk to which was added one of the constituents of ordinary synthetic basal rations, such as casein, agar, starch, yeast, salts, cod liver oil, wheat germ oil, gelatin, and rice polishings. This procedure resembles somewhat that followed by Mattill and coworkers (3), but these investigators were concerned primarily with reproduction. Since vitamin D may be considered a constituent of a basal ration, one group of rats was fed milk irradiated in a thin layer under a Cooper-Hewitt lamp for ten minutes at a distance of 14 inches from the burner. Two male and two female rats were placed together in a cage in order to allow every opportunity for reproduction to occur. The amount of each supplement fed, arbitrarily chosen, was weighed or measured each day and added to the milk. This was thoroughly stirred in glass beakers and placed in the cages in the afternoon. The amount of milk fed at this time was small enough to insure complete consumption overnight. In the morning more milk was added to

---

* Received for publication April 25, 1929.

TABLE 1

Gain in weight and hemoglobin content of the blood of rats fed milk only, and milk plus various supplements

RAT NUMBER	INITIAL WEIGHT	AFTER 2 MONTHS		AFTER 3 MONTHS	
		Weight	Hemoglobin	Weight	Hemoglobin
Milk exclusively (ad lib.)					
	grams	*grams*	*grams per 100 cc.*	*grams*	*grams per 100 cc.*
E135 ♂	59	90	1.38−	*	*
E136 ♂	60	95	1.38−	Dead	
E137 ♀	64	95	1.38−	*	*
E138 ♀	58	96	2.34	Dead	
Milk plus dried yeast (0.4 gram per rat)					
E139 ♂	61	155	16.52	211	16.52
E140 ♂	62	183	15.84	233	15.84
E141 ♀	62	123	16.52	152	16.52
E142 ♀	60	148	13.77	172	13.77
Milk plus cod liver oil (3 drops per rat)					
E143 ♂	63	137	2.07	Dead	
E144 ♂	61	Dead			
E145 ♀	61	85	2.75	Dead	
E146 ♀	56	86	2.62	Dead	
Milk plus McCollum's salt mixture 185 (0.25 gram per rat)					
E155 ♂	60	93	8.68	134	13.08
E156 ♂	61	152	12.39	177	11.70
E157 ♀	56	117	16.52	126	16.52
E158 ♀	62	117	12.39	137	12.67
Milk plus gelatin (0.25 gram per rat)					
E167 ♂	52	150	2.75	169	2.34
E170 ♂	54	130	2.34	Dead	
E168 ♀	52	138	3.86	150	3.86
E169 ♀	49	Dead			
Milk plus agar (0.50 gram per rat)					
E175 ♂	52	180	9.36	214	7.57
E178 ♂	52	160	6.75	170	9.36
E176 ♀	50	133	8.12	135	4.96
E177 ♀	49	128	7.16	135	10.33

TABLE 1—*Concluded*

RAT NUMBER	INITIAL WEIGHT	AFTER 2 MONTHS		AFTER 3 MONTHS	
		Weight	Hemoglobin	Weight	Hemoglobin
Milk plus casein (2 grams per rat)					
	grams	*grams*	*grams per 100 cc.*	*grams*	*grams per 100 cc.*
E180 ♂	64	174	13.08	190	15.15
E182 ♂	52	195	13 77	205	11.15
E179 ♀	59	134	13.77	145	16.25
E181 ♀	49	138	15.15	Died from loss of blood	
Milk plus starch (2 grams per rat)					
E185 ♂	51	175	8.26	218	10.60
E186 ♂	49	157	7.30	.90	11.02
E183 ♀	50	130	8.40	158	10.88
E184 ♀	49	135	7.57	158	13.77
Milk plus rice polishings (0.4 gram per rat)					
E247 ♂	51	144	3.17	168	2.62
E249 ♂	54	146	2.34	150	1.79
E246 ♀	51	117	5.23	129	5.92
E248 ♀	53	Dead			
Milk plus wheat germ oil (3 drops per rat)					
E251 ♂	62	Dead			
E253 ♂	58	88	1.38	Dead	
E250 ♀	55	65	3.03	Dead	
E252 ♀	48	Dead			
Milk, irradiated (ad lib.)					
E238 ♂	52	72	1.38	Dead	
E240 ♂	50	71*	1.38*	*	*
E239 ♀	52	89	2.48	Dead	
E241 ♀	47	106*	1.38*	*	*

* Yeast added.

the beakers, so that the consumption of milk was ad libitum. Weights were recorded weekly and hemoglobin determinations were made on one rat of each group each week. The technique of obtaining blood samples and of determining hemoglobin has been previously described (2).

RESULTS

The results are presented in table 1.

It is very evident that yeast, McCollum's salt mixture 185, agar, casein and starch were helpful in preventing anemia. Of these substances, yeast was by far the most effective, the hemoglobin of the rats receiving it remaining at a high level throughout. In the case of the other supplements there was a drop in hemoglobin during the critical period, but as the rats matured there was gradual regeneration.

A special word of explanation concerning the yeast used needs to be made at this point. The sample was a dry, starch-free powder, originally purchased for use as a source of vitamin B. The amount fed, 0.4 gram, had been found to be adequate to allow normal growth on an otherwise vitamin-B-free diet. The results obtained with this yeast, both as a curative and as a preventive of nutritional anemia, were so successful that two other samples from the same company, and seven samples from different companies, were assayed, for their anti-anemic potency. Each sample was found to be decidedly inferior to the first one tried. This is emphasized here because it points out the lack of uniformity that may exist in even a high-grade product, and also it suggests a precaution to be taken when formulating a basal ration to be used in connection with iron metabolism studies.

TABLE 2

Iron and copper content of supplements, and daily intake

MATERIAL	IRON	COPPER	DAILY INTAKE	
			Fe	Cu
	per cent	*per cent*	*mgm.*	*mgm.*
Yeast	0.0918	0.0032	0.49	0.04
Salts 185	0.2561		0.76	0.03
Agar	0.0175		0 21	0.03
Casein	0.0162	0.0024	0.44	0.07
Starch	0.0135	0.00073	0.39	0.04

In order to find a possible explanation of the behavior of these substances in preventing anemia, determinations were made of their copper and iron content. Copper was determined by the Biazzo method (4) and iron by the Fowweather (5) method, except in the case of the salt mixture where the Elvehjem and Lindow method for copper (6), and the Elvehjem and Hart method for iron (7) were used. These figures are presented in table 2.

This indicates a rather definite relationship between the amount of copper and iron required by the rat for normal hemoglobin formation. Wherever at least 0.45 mgm. of iron, and 0.04 mgm. of copper were furnished, the hemoglobin level was highest, as in the case of yeast and casein. Hart, Steenbock, Waddell and Elvehjem (8) secured excellent results through the administration six times per week of 0.05 mgm. of copper and 0.5 mgm. of iron.

DISCUSSION

The exceptional response obtained through supplementing milk with 0.4 gram of yeast daily may be attributed to an adequate intake of copper and iron. The amounts of copper and iron supplied were probably optimum, the ratio between these two elements being 1:12.25. That no organic factor was involved was shown when the ash of 0.4 gram of yeast was used with equally good results. Evidently vitamin B was no factor as negative results were obtained with rice polishings. The same applies to vitamins A, D, and E, as cod liver oil, irradiated milk and wheat-germ oil likewise exerted no influence. Cartland and Koch (9) concluded that vitamins A, B, and E probably were not essential for hemoglobin formation in the rat. These same investigators found that casein promoted hemoglobin synthesis, as did McCay (10).

The weights at two and three months (table 1) indicate that when rats are given whole milk ad libitum, the only deficiency exists in the mineral portion of the diet. When additional protein and energy in the form of casein and starch, respectively, were added, no increase in the rate of growth occurred. Of the vitamins, B and E might have been slightly deficient. The inferior growth of rats receiving the salt mixture, however, points to a mineral deficiency. The rats in this group received an adequate supply of iron as iron citrate, but their intake of copper was inadequate. The addition of bulk in the form of agar had no effect on the rate of growth and was not very effective in preventing anemia because at the level fed the total intake of copper and iron was insufficient.

Yeast, casein, agar, starch and McCollum's salt mixture 185 are often used in synthetic basal rations. When iron metabolism, or hemoglobin regeneration studies are conducted, and a basal ration containing any of the above ingredients is contemplated, a careful evaluation of both the copper and iron content of the diet should be made.

SUMMARY

Yeast, casein, starch, agar, and McCollum's salt mixture 185 were found effective in preventing nutritional anemia in rats fed an exclusive whole milk diet. The degree of effectiveness was found to depend in general upon the amount of copper and iron furnished by each substance.

Cod liver oil, gelatin, rice polishings, wheat germ oil, and irradiated milk were found to be ineffective in preventing nutritional anemia in rats.

An exclusive whole milk diet fed ad libitum to rats furnishes adequate protein, energy, and vitamins for growth, but is deficient in copper and iron.

REFERENCES

(1) Waddell, J., Steenbock, H., Elvehjem, C. A., and Hart, E. B. 1928 Iron in nutrition. V. The availability of the rat for studies in anemia. Jour. Biol. Chem., lxxvii, 769–775.

(2) Krauss, W. E. 1929 Studies on the nutritive value of milk. I. The production of nutritional anemia in albino rats through exclusive whole milk diets. Jour. Dairy Sci., xii, 74–79.

(3) Mattill, H. A., and Conklin, R. C. 1920 The nutritive properties of milk with special reference to reproduction in the albino rat. Jour. Biol. Chem., xliv, 137–158.

(4) Biazzo, R. 1926 Metodo rapido di determinazione del rame nelle conserve di prodotti vegetali rinverditi. Annali di Chimica Applicata, xvi, Fasc. 2.

(5) Fowweather, F. S. 1926 The determination of iron in blood, tissues and urine. Biochem. Jour., xx, 93–98.

(6) Elvehjem, C. A., and Lindow, C. W. 1929 The determination of copper in biological materials. Jour. Biol. Chem., lxxxi, 435–443.

(7) Elvehjem, C. A., and Hart, E. B. 1926 Iron in nutrition. II. Quantitative methods for the determination of iron in biological materials. Jour. Biol. Chem., lxvii, 43–51.

(8) Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A. 1928 Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. Jour. Biol. Chem., lxxvii, 797–812.

(9) Cartland, G. F., and Koch, F. C. 1928 Diet proteins and vitamins as related to hemoglobin formation in the rat. Amer. Jour. Physiol., lxxxvii, 249–261.

(10) McCay, C. M. 1928 The influence of protein, blood, liver, fat, iron and potassium in the diet upon the rate of blood regeneration after hemorrhage in the rat and dog. Amer. Jour. Physiol., lxxxiv, 16–35.

THE PHYSIOLOGICAL EFFECT OF RATIONS RESTRICTED PRINCIPALLY OR SOLELY TO THE ALFALFA PLANT

I. THE CALCIUM, PHOSPHORUS AND NITROGEN METABOLISM OF DAIRY CATTLE*

J. R. HAAG AND J. S. JONES

Department of Agricultural Chemistry

AND

I. R. JONES AND P. M. BRANDT

Department of Dairy Husbandry
Oregon Agricultural Experiment Station, Corvallis, Oregon

Due to the rather common practice in many of our western alfalfa producing sections of feeding dairy cattle exclusively or almost so on alfalfa, the Department of Dairy Husbandry of the Oregon Experiment Station (1) has been conducting practical alfalfa feeding trials in an attempt to determine the economy of this practice and the extent to which alleged regional differences exist in the quality of alfalfa hay. During the summers of 1926[1] and 1927 animals from this group were placed in metabolism stalls and their calcium, phosphorus and nitrogen balances were determined for periods during which the daily milk yields varied from approximately 9 to 18 kgm. In these metabolism studies, we fed alfalfa hay with and without supplements of starch, linseed oil meal, or di-sodium phosphate.

The study of rations restricted to alfalfa is of peculiar interest in extensive regions of the West where alfalfa is grown so abundantly and cheaply that the relatively high price of concentrates has led to the frequent practice of restricting dairy cattle very

---

* Received for publication May 2, 1929. Published with the approval of the Director.

[1] The 1926 trials were started and partially completed while the nutrition work in the Department of Agricultural Chemistry was in charge of Dr. H. G. Miller. A large share of the analytical work reported in this paper was done with the assistance of Mr. H. M. Shepardson.

largely to alfalfa. Furthermore, this problem is of interest to the nutrition worker because it deals with an attempt to determine what substances, in relatively small amounts, are needed to make a fairly well balanced ration from a common leafy leguminous plant. Among the factors to be considered in rations restricted largely to alfalfa hay are: their net energy value, the biological value of alfalfa proteins, the phosphorus content of alfalfa, and the fact that alfalfa may contain anywhere from about 3 to 25 times as much calcium as phosphorus. The last mentioned fact appears to have been almost entirely overlooked by nutrition workers, as has also the fact that alfalfa hay frequently contains less than 0.20 per cent phosphorus.

It is not our purpose here to review the extensive literature dealing with experiments in which alfalfa has been fed as a constituent of more or less balanced rations. Experimental data dealing with alfalfa hay as the sole or principal constituent of rations for dairy cattle are limited, presumably because the practical aspects of this problem have not impressed most investigators. Attention, however, is called to the work of Woll (2) and to the discussions of Eckles (3) and of Henry and Morrison (4), dealing with the economy of alfalfa hay feeding. It should be kept in mind that the problem varies with the quality of hay and with the amount of alfalfa which is fed in the green state.

THE 1926 METABOLISM TRIALS

In these trials we fed second cutting alfalfa hay with and without supplements of starch or linseed oil meal. The hay was taken from the same lots as that used in the practical feeding trials and had been selected as being representative of the regions in which it was grown. This hay (except that fed to cow 629) was grown under irrigation in eastern Oregon and was cut when in about one-half bloom. It was clean and well cured but was slightly coarse. Cow 629 was fed quite a coarse grade of hay grown in the Willamette Valley. The nitrogen content of the eastern Oregon hay varied from 1.58 to 1.71 per cent; the calcium content from 0.92 to 1.03 per cent; and the phosphorus content

TABLE 1

Description and history of the cows used in the 1926 metabolism trials

COW NUMBER	BREED	APPROXIMATE AGE	LACTATION PERIOD	PREVIOUS FEEDING HISTORY
222	Holstein	8	Fifth period began May 13, 1926	On alfalfa hay from February 1, 1926
468	Ayrshire	3½	Second period began March 22, 1926	On alfalfa hay from February 1, 1926
623	Guernsey	6½	Fifth period began March 10, 1926	On alfalfa hay from April 7 to July 8, 1924. On alfalfa hay from January 24, 1925
629	Guernsey	5	Third period began March 10, 1926	On alfalfa hay from February 1, 1926

TABLE 2

Average daily metabolism data secured from 1926 trials

COW NUMBER	DATE	DISTINGUISHING FEATURES OF RATIONS USED	MILK YIELD	NITROGEN Feed Milk Excreta Balance	CALCIUM Feed Milk Excreta Balance	PHOSPHORUS Feed Milk Excreta Balance
			kgm.	*grams*	*grams*	*grams*
623	April 7–17	13.40 kgm. alfalfa hay	12.57	226.6	132.9	23.5
				65.5	17.1	11.1
				166.8	88.6	14.0
				−5.7	+27.2	−1.6
468	April 7–17	14.26 kgm. alfalfa hay	16.58	240.8	142.7	25.0
				88.0	16.5	11.6
				178.2	109.9	14.4
				−25.4	+16.3	−1.0
623	May 14–24	9.40 kgm. alfalfa hay; 2.72 kgm. starch	9.29	161.0	97.0	16.5
				41.8	11.8	10.4
				125.6	98.8	16.2
				−6.4	−13.6	−10.1
468	May 14–24	11.75 kgm. alfalfa hay; 2.72 kgm. starch	14.52	201.2	121.0	20.6
				69.2	16.0	13.8
				145.7	76.9	19.6
				−13.7	+28.1	−12.8

from 0.162 to 0.175 per cent. The nitrogen content of the Willamette Valley hay varied from 1.26 to 1.54 per cent; the calcium content from 0.81 to 1.14 per cent; and the phosphorus content from 0.185 to 0.212 per cent. The nitrogen content of the hay fed to cow 629 during the metabolism trials proved to be unexpectedly low. Previous analysis of samples taken from the same lot from which this hay was obtained had shown a nitrogen content similar to that of the eastern Oregon hay. The crude fiber content of the above hays varied from 30.0 to 33.0 per cent.

TABLE 3

Average daily metabolism data secured from 1926 trials (Continued)

COW NUMBER	DATE	DISTINGUISHING FEATURES OF RATIONS USED	MILK YIELD	NITROGEN Feed Milk Excreta Balance	CALCIUM Feed Milk Excreta Balance	PHOSPHORUS Feed Milk Excreta Balance
			kgm.	*grams*	*grams*	*grams*
222	June 21–July 1	16.66 kgm. alfalfa hay	18.80	253.9	147.0	26.6
				77.5	13.4	14.7
				193.2	111.1	22.0
				−16.8	+22.5	−10.1
629	June 21–July 1	11.28 kgm. alfalfa hay	10.07	139.5	85.3	26.7
				52.6	8.0	11.9
				119.0	97.7	16.2
				−32.1	−20.4	−1.4
222	August 25–September 4	17.90 kgm. alfalfa hay; 1.36 kgm. linseed oil meal	14.82	347.7	163.1	41.1
				68.3	14.3	11.7
				293.0	145.4	35.3
				−13.6	+3.4	−5.9
629	August 25–September 4	12.84 kgm. alfalfa hay; 1.36 kgm. linseed oil meal	8.12	265.4	146.7	35.8
				43.8	8.4	9.0
				213.0	109.8	32.4
				+8.6	+28.5	−5.6

The cows were placed in metabolism stalls and subjected to a preliminary feeding period while becoming accustomed to the daily routine involved in metabolism trials. The mixed excreta were collected, weighed and sampled daily. As a general rule, we conducted our work in units of five days each in order to avoid the chance of losing the results of an entire period. While we report our results for the entire period, it may be remarked that we occasionally found the results of a five-day period out of line, indicating that even for such a uniform ration as chopped alfalfa hay, metabolism periods should be of at least ten days duration.

The cows used in the 1926 trials are briefly described in table 1. The results obtained with two cows fed alfalfa hay with and without the addition of starch are reported in table 2; those obtained with two cows fed alfalfa hay with and without the addition of linseed oil meal, in table 3.

Upon examining the balance data in tables 2 and 3, we were at once impressed with the fact that the phosphorus balances were always negative and the nitrogen balances usually so. In view of the high calcium intake one would expect to find the calcium balances positive, although it is not clear why they should bear so little apparent relation to the losses of phosphorus from the body.

The supplements of starch and linseed oil meal did not lead to results from which definite conclusions can be drawn. The feeding of starch resulted in a decreased consumption of hay, while the feeding of linseed oil meal was accompanied by a slight increase in hay consumption. We are therefore unable, from these experiments, to draw any definite conclusions concerning the effect of an increase in total digestible nutrients over those contained in rations restricted to alfalfa hay, nor are we able to make any definite statements concerning the value of supplementing alfalfa hay with linseed oil meal proteins, since the latter were added to an already high level of protein intake and could not therefore be expected to show marked results unless the supplementary effect was very pronounced.

A comparison of the actual hay consumption and milk yields with the commonly accepted feeding standards, compels us to come to the conclusion that the intake of total digestible nutrients and of phosphorus can not be considered adequate for more than very moderate milk production. The faulty nitrogen assimilation may be related to one or more of a number of factors, among which are the energy and protein intakes, the biological value of alfalfa protein, a possible relationship between phosphorus and protein metabolism and the general suitability of rations restricted to alfalfa hay. In the case of the 1926 trials the low protein content of the hay undoubtedly was an important factor contributing to the negative nitrogen balance.

During the course of the above experiments, cows 468 and 623 lost approximately 25 to 30 pounds in live weight. Cows 222 and 629 suffered somewhat greater losses in weight during the periods on alfalfa hay only, but remained practically constant in weight during the periods when alfalfa hay was supplemented with linseed oil meal. The losses in weight referred to are considered as being incidental to the stage of lactation and quite independent of the various supplements used in our balance trials. It has been our experience that cows fed exclusively on alfalfa hay begin their lactation period in good condition with a fairly liberal milk flow, which is followed by a sharp decline in milk flow and a moderate loss in body weight.

THE 1927 METABOLISM TRIALS

On the basis of the preliminary results secured from the 1926 metabolism trials and certain theoretical considerations, such as the significance of the Ca/P intake ratio, we outlined in 1927 a more extensive program for the study of rations restricted principally or solely to alfalfa. The 1927 trials were conducted as part of a series which we hope will throw some light on the question of the significance of the Ca/P intake ratio in the nutrition of dairy cattle. No extensive search of the literature is necessary to show that alfalfa hay may contain anywhere from about 3 to 25 times as much calcium as phosphorus, although under ordinary conditions this ratio is more likely to be around 5 to 8. For example, Scott (5) has recently called attention to a sample of alfalfa hay which contained 28 times as much calcium as phosphorus.

The 1927 trials were conducted in much the same manner as those in 1926. Two cows were fed alfalfa with and without the addition of di-sodium phosphate ($Na_2HPO_4 \cdot 12H_2O$). After a preliminary feeding period in the metabolism stalls, a ten-day balance was run with alfalfa only, followed by an intermediate period of four days, during which the sodium phosphate was fed in amounts increasing from 200 to 400 grams daily. During the next ten days 400 grams were fed daily. The behavior of the cows in the stalls and the collection of excreta were entirely satisfactory. We made a practice of exercising the cows about 15 minutes each day while the excreta were being weighed, mixed and sampled.

The hay used in these trials was second cutting grown on non-irrigated land. It was cut at about one-fourth bloom, left in the swath for one-half day, in the windrow for one-half day, shocked for 7 days and stacked. The hay was graded as U. S. No. 1, and was finer in texture and more leafy than that used in the 1926 trials. This hay contained 2.01 per cent nitrogen, 1.37 per cent calcium, 0.174 per cent phosphorus, and 27.6 per cent crude fiber. The cows used in these trials are briefly described in table 4.

TABLE 4

Description and history of cows used in the 1927 metabolism trials

COW NUMBER	BREED	APPROXIMATE AGE	LACTATION PERIOD	PREVIOUS FEEDING HISTORY
		years		
456	Ayrshire	5½	Fourth period began May 5, 1927	On alfalfa hay from January 24, 1925
462	Ayrshire	5⅓	Fourth period began April 28, 1927	On alfalfa hay from January 24, 1925

In table 5 are given the data secured in the 1927 trials. We have included the four-day intermediate period because it forms a part of the picture of what took place during this period. In contrast with the 1926 trials, the nitrogen balances were positive. This appears to have been due to the difference in nitrogen content of the hays used. The 1926 hay analyzed from approximately 1.3 to 1.7 per cent nitrogen, while that of 1927 analyzed

2.0 per cent nitrogen. The calcium balances were again positive. As before, when alfalfa alone was fed, the phosphorus balances were negative. When, however, di-sodium phosphate was fed, slightly positive balances were obtained. We realize that the

TABLE 5

Average daily metabolism data secured from 1927 trials

COW NUMBER	DATE	DISTINGUISHING FEATURES OF RATIONS USED	MILK YIELD	NITROGEN Feed Milk Excreta Balance	CALCIUM Feed Milk Excreta Balance	PHOSPHORUS Feed Milk Excreta Balance
			kgm.	*grams*	*grams*	*grams*
456	July 7–17	14.18 kgm. alfalfa hay	15.61	284.7	194.3	24.6
				66.6	15.3	12.8
				214.3	172.0	15.9
				+3.8	+7.0	−4.1
462	July 7–17	14.23 kgm. alfalfa hay	12.59	286.5	195.5	24.8
				56 6	14.2	10.4
				209.7	173.3	16.2
				+20.2	+8.0	−1.8
456	July 17–21	13.57 kgm. alfalfa hay; 200, 300, 390 and 400 grams, respectively of di-sodium phosphate	14.64	272.5	186.0	52.3
				62.5	15.0	11.5
				193.3	157.0	24.0
				+16.7	+14.0	+16.8
462	July 17–21	13.42 kgm. alfalfa hay; 200, 300, 395 and 400 grams, respectively of di-sodium phosphate	12.24	271.3	184.8	52.0
				52.0	13.8	9.8
				203.8	151.3	23.8
				+15.5	+19.7	+18.4
456	July 21–31	13.43 kgm. alfalfa hay; 400 grams daily of di-sodium phosphate	13.94	269.9	183.9	58.4
				56.3	15.3	11.1
				202.4	164.5	46.0
				+11.2	+4.1	+1.3
462	July 21–31	13.57 kgm. alfalfa hay; 400 grams daily of di-sodium phosphate	10.81	273.3	186.6	58.8
				46.1	11.8	8.5
				204.1	167.4	48.4
				+23.1	+7.4	+1.9

differences between the negative balances on hay alone and the positive balances when sodium phosphate was fed, are not large, but they occurred so consistently that they appear to be significant.

Attention is called to the data during the four-day intermediate period. It will be noted that the phosphorus lag in the digestive tract shows up very nicely in an apparently high positive balance, and that along with it there is evidence of an associated lag in the excretion of calcium. As would be expected, the nitrogen balance data are not affected in this manner. Our results are in agreement with those of Meigs et al. (6) in that they indicate about a two-day lag in the excretion of phosphorus.

A DISCUSSION OF THE SIGNIFICANCE OF OUR RESULTS

It is difficult to determine to what extent dairy cattle restricted largely to alfalfa hay are merely suffering from under-feeding due to the bulky nature of the ration and to what extent specific nutritive factors are involved. We are reasonably certain that production must often be limited by the intake of total digestible nutrients, and in the case of low protein hay, by the protein intake. The milk production of the animals restricted to alfalfa hay was approximately one-half that to be expected on the regular herd ration. When hay of unusual quality and green alfalfa are fed, it is reasonable to expect somewhat higher production. It is our feeling that there is still a great deal to be learned concerning the factors which determine "quality" of hay.

Our data on nitrogen assimilation do not warrant any definite conclusions concerning the biological value of alfalfa protein. We were at first inclined to interpret the negative nitrogen balances obtained during 1926 as evidence of a lack of protein quality, but our 1927 data do not necessarily support such an interpretation. We (7) are, however, accumulating evidence with rats which shows that alfalfa protein (as supplied by finely ground leaves at a level of 12 to 15 per cent crude protein) does not constitute an adequate source of cystine.

Standards for the phosphorus requirements of dairy cattle are so limited that it is difficult to definitely evaluate rations restricted largely to alfalfa from the point of view of their phosphorus content. The regularity with which we obtained negative phosphorus balances on alfalfa hay, however, indicates that the phosphorus content of alfalfa hay can not be considered adequate for more than moderate milk production. We consider it worthwhile to emphasize the fact that alfalfa hay may often contain less than 0.20 per cent phosphorus, and therefore should not be regarded as a phosphorus-rich feed. Although we have some evidence originating with practical dairymen, indicating a phosphorus deficiency among animals restricted largely to alfalfa, the situation in Oregon does not appear to have reached an acute stage. It is our opinion that this is true largely because even most well-bred animals lose their impulse to secrete milk to a sufficient extent to protect their body stores of phosphorus against loss during most of their lactation cycle.

The data presented in this paper do not indicate to what extent phosphorus retention is conditioned by the high Ca/P intake ratios incident to rations restricted largely to alfalfa. Meigs and coworkers (6) have expressed the opinion that there is some evidence which indicates that rations for dairy cattle should preferably contain not more than two times as much calcium as phosphorus. In a later paper (8) it is suggested that "the amount of calcium probably should not exceed the amount of phosphorus by more than 50 per cent." While general considerations compel us to regard extremely high Ca/P intake ratios with suspicion, we must admit that our present data may also be interpreted as indicating that such ratios may be quite high without producing nutritional disaster.

In addition to the metabolism trials reported in this paper, we conducted another series during the summer of 1928, again using cows 456 and 462. We repeated the feeding of alfalfa hay with and without di-sodium phosphate, and, in addition, we fed alfalfa hay with supplements of calcium carbonate or bone flour in an attempt to obtain further information on the significance of the Ca/P intake ratio. The results obtained from this series of trials will be available in the near future.

SUMMARY

We have reported a series of metabolism studies in which the nitrogen, calcium and phosphorus balances of dairy cattle were determine for 12 ten-day periods, during which the daily milk yields varied from approximately 9 to 18 kgm. The rations fed consisted of alfalfa hay with and without supplements of starch, linseed oil meal, or di-sodium phosphate.

According to our data, cows in the early lactation period were usually in positive calcium balance; in negative nitrogen balance when the hay contained less than 2.0 per cent nitrogen; and always in negative phosphorus balance when only alfalfa hay was fed. Feeding di-sodium phosphate changed the negative phosphorus balances to slightly positive balances.

We have also included a general discussion of 'some of the factors which may be involved in rations restricted principally or solely to alfafa.

REFERENCES

(1) Director's Biennial Report, 1924–1926, Oregon Agr. Exp. Sta., 41–43.

(2) Woll, F. W. 1918 Alfalfa as a sole feed for dairy cattle. Jour. Dairy Sci., i, 447–461.

(3) Eckles, C. H. 1923 Dairy Cattle and Milk Production. New York, Revised ed., 591 p.

(4) Henry, W. A., and Morrison, F. B. 1927 Feeds and feeding. Madison, Wisc., ed. 19, 770 p.

(5) Scott, S. G. 1929 Phosphorus deficiency in forage feeds of range cattle. Jour. Agric. Rsh., xxxviii, 113–130.

(6) Meigs, E. B., Turner, W. A., Harding, T. S., Hartman, A. M., and Grant, F. M. 1926 Calcium and phosphorus metabolism in dairy cows. Jour. Agric. Rsh., xxxii, 833–860.

(7) Director's Biennial Report, 1926–1928, Oregon Agr. Exp. Stat., 79.

(8) Turner, W. A., Harding, T. S., and Hartman, A. M. 1927 The relative assimilation by dairy cows of clover and alfalfa hays and of rations of different calcium and phosphorus content. Jour. Agric. Rsh., xxxv, 625–635.

THE CALCULATION OF ABSOLUTE VISCOSITY FROM DATA SECURED BY MEANS OF THE TORSION PENDULUM VISCOSIMETER*

RANDALL WHITAKER† AND B. L. HERRINGTON

Department of Dairy Industry, Cornell University, Ithaca, N. Y.

The Mojonnier-Doolittle viscosimeter is an instrument of the torsion pendulum type, designed for determining the viscosity of dairy products. This viscosimeter has found favor because of the ease and rapidity of operation, and simplicity of construction. It has the disadvantage, however, that the unit of measurement termed the degree of retardation, is relative rather than absolute. It has been impossible to compare data obtained with one of these instruments with readings obtained with another of this or any other type of viscosimeter. The authors have developed a method of converting degrees retardation, as measured by the Mojonnier-Doolittle viscosimeter into centipoise, the unit of absolute viscosity.

DESCRIPTION AND OPERATION OF THE TORSION PENDULUM VISCOSIMETER

The torsion pendulum was first used for the study of viscosity by Coulomb (1) in 1800. Babcock (2) in 1886 used a similar instrument for the study of the viscosity of milk, and Doolittle (3) in 1893 recommended that the instrument be used for the examination of oils. Babcock reported his viscosities in terms of standard sucrose solutions and this method was adopted by Doolittle (4).

More recently the instrument has been marketed by the Mojonnier Brothers Company under the name of the Mojonnier-Doolittle viscosimeter, and it has been widely used by workers in the dairy industry. That the present day "Mojonnier-Doolittle" instrument is still almost identical with the original

---

* Received for publication May 2, 1929

† Supported by a grant from American Dry Milk Institute.

viscosimeter used by Coulomb one hundred and thirty years ago may be seen from his drawing which is reproduced in figure 1.

A detailed description of the Mojonnier-Doolittle viscosimeter and its operation is given by Mojonnier and Troy (5). The general principle of the viscosimeter is the measurement of the resistance offered by a liquid to a torsion pendulum which is immersed in that liquid, the resistance or retardation effect being dependent upon the viscosity of the liquid. The sphere, which is suspended by a wire, is caused to oscillate by releasing it after it has been twisted through exactly one revolution. Upon being released, the sphere makes one complete and part of a second revolution, finally coming to a stop and then revolving in the opposite direction.

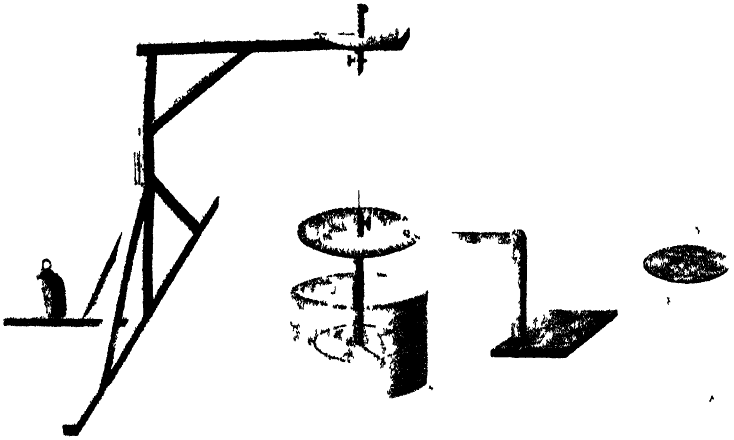


FIG. 1 THE ORIGINAL TORSION PENDULUM VISCOSIMETER DESCRIBED BY COULOMB IN THE YEAR 1800

That part of a revolution by which the pendulum fails to ascend to its original degree of twist, when measured in degrees, is recorded as degrees retard. It may be read directly from a dial attached to the upper part of the pendulum.

DEVELOPMENT OF THE EQUATION

In order to discuss the motion of the viscosimeter ball, it will be convenient to designate certain things by symbols. Let us, therefore, represent the point of rest of the torsion pendulum as O, the starting point of the ball $-A$ and consequently the position which would be reached by the ball if there were no retarding force would be $+A$. The point actually reached by the ball on the first swing will be indicated as X, and the distance between X and A (called the retard) may be designated as R. Angular distances from the position of rest (O) may be designated by the letter S, or by a combination of symbols designating the points concerned. The viscosity of the material under investigation will be represented by V. The letter K with suitable subscripts will be used for various constants which will appear.

When the ball of the viscosimeter is rotated to position $-A$, a certain amount of potential energy will be stored in the wire. When the ball is released, this energy will be converted into kinetic energy as the ball passes from $-A$ to O, while from O to A the kinetic energy will again be transformed into potential energy. At the turning point the kinetic energy will have fallen to zero; and if no energy has been lost while the ball is in motion, the turning point will be at $+A$ where the potential energy is exactly equal to the potential energy at $-A$. In general, this will not be the case. Energy will have been lost from the system and the turning point will be at X instead of A. The difference between the potential energies of the positions A and X will be exactly equal to the energy lost from the sphere in passing from $-A$ to X. This difference in energy may be represented by the definite integral $K_1\int_{OX}^{OA} SdS$ which on integration gives the difference in energy as equal to $K_2\,(\overline{OA}^2 - \overline{OX}^2)$. It must be assumed throughout the discussion that the twist imparted to the wire at positions $-A$ and A is not great enough to alter the position of rest of the ball.

The energy lost from the torsion pendulum may, for convenience, be divided into two parts; the first part will be used up in overcoming the internal resistance in the wire and the viscosity of the air; the amount of energy lost in this way will usually be small and may be considered as proportional to the square of the distance traversed by the pendulum $(\overline{OA} + \overline{OX})$. The second, and larger part will be lost in overcoming the viscous resistance of the material under investigation.

The energy required to move a surface through a viscous medium is proportional to the viscosity of the medium, the effective area of the surface, the distance traversed, the velocity of the surface with respect to the liquid and is an inverse function of the distance to the opposite wall. For a given viscosimeter ball centered in the same cup which is filled to the same depth each time, the surface area and the distance to the walls of the cup will be constant; therefore, the above reduces to $K_3 \cdot V \cdot$ velocity $\cdot$ distance. The distance traversed by the ball is $(\overline{OA} + \overline{OX})$. For small values of viscosity the pendulum will obey, approximately, the laws of harmonic motion and its mean velocity will be equal to $K_4 (\overline{OA} + \overline{OX})$. Substituting these values in the above equation, we find that the work done by the pendulum is $K_5 \cdot V \cdot (\overline{OA} + \overline{OX})^2 + K_6 (\overline{OA} + \overline{OX})^2$ where the last term represents the energy lost in the wire and air.

Equating the energy lost from the pendulum to the work done, we obtain:

$$K_2 (\overline{OA}^2 - \overline{OX}^2) = K_5 \cdot V \cdot (\overline{OA} + \overline{OX})^2 + K_6 (\overline{OA} + \overline{OX})^2$$

which reduces to

$$V = \frac{K(\overline{OA} - \overline{OX})}{(A + X)} - K_6$$

The equation may be simplified to the following form:

$$V = \frac{K \cdot R}{(2\,\overline{OA} - R)} - K'$$

DISCUSSION OF EQUATION

In the derivation of this equation, several assumptions have been made which limit its application. The errors introduced by these assumptions should be understood before attempting to apply the equation.

It was first assumed that the energy lost in the internal resistance of the wire and the resistance of the air was proportional to the square of the amplitude of the swings. That this is justified was shown by the experiments of Coulomb (1), who states that he found "la diminution de l'amplitude à chaque oscillation resultante de cette imperfection (imperfect elasticity of the wire, and air resistance), etoit toujours proportionelle à l'amplitude des oscillations" (page 266).

A second assumption, and the most likely of all to be in error, is that the restraining force is proportional to the first power of the velocity. A full expression for the relation between the retarding force and the velocity would contain at least two terms; the first containing the velocity to the first power, and the second containing the velocity to the second power. For very low velocities, the second term may be neglected as was shown by Coulomb in the article cited; at higher velocities, the first term becomes negligible in comparison with the second term and many texts merely state that the retarding force is proportional to the square of the velocity. In the above equation, it is assumed that the velocity is low enough to safely neglect the second term. In the case of the Mojonnier-Doolittle instrument there may be some question as to the validity of this assumption, since the speed is much higher than was recommended by Coulomb and by Babcock. The error introduced in this way will be greater in the case of liquids of low viscosity and may entirely prevent the application of this equation to the instrument provided with a large viscosimeter ball.

Another assumption was made in deriving the equation: that the period of oscillation remains constant regardless of the viscosity. This is obviously not true but the deviation for the normal range of retard values is very small. It may be calculated and shown that for a retard of 120° the error is less than 1 per cent.

The assumptions regarding the constancy of the dimensions of the instrument are obviously under the control of the operator and need no comment.

This equation has doubtless been used by other workers. The relations involved have been known for a long time, but the usual form of equation which is given is not applicable to the single observation recorded as degrees retard. In any case, the relation does not seem to be generally known to the users of the Mojonnier-Doolittle viscosimeter.

TABLE 1

Determination of constant K and calculation of absolute viscosity from degrees retardation of five standard oil samples

OIL SAMPLE NUMBER	(1) OBSERVED RETARD	(2) $\frac{R}{720 - R}$	(3) KNOWN VISCOSITY	(4) K	(5) CALCULATED VISCOSITY	(6) ERROR	(7) ERROR
	degrees		*cp*		*cp*	*cp*	*per cent*
1	16	0 0227	19	1,806	19.61	+0.61	+3.21
2	28.5	0 0412	55	1,869	53 52	−1.48	−2.69
3	72	0 1111	186	1,872	181 6	−4.4	−2.36
4	112	0.1842	313	1,816	315.6	+2.6	+0.83
5	216	0.4285	751	1,803	763.4	+12.4	+1.65
				Mean 1,833			Mean deviation 2.14

THE VALIDITY OF THE EQUATION AND THE DETERMINATION OF THE CONSTANTS

In order to determine if the equation, based on the assumptions discussed above, is applicable to the Mojonnier-Doolittle viscosimeter, experimental work was performed. For this purpose five oils of known absolute viscosity were obtained. Table 1 gives the viscosity of these oils at a given temperature in centipoise, as determined by the Bureau of Standards; and the viscosity expressed in degrees retardation, as measured by the Mojonnier-Doolittle viscosimeter. In figure 2, the known values of viscosity were plotted against the experimental values of $\frac{R}{2A - R}$. By extrapolating to zero retard, the value of $-K'$ was found to be −22. This value of K' was then substituted in the original equation and then the data for each of the five oils were used to calculate the values of the constant K. These values are given in column 4. The mean value of the constant K was then used to calculate backward and determine the apparent viscosity of each of the five samples. These values are tabulated in column 5, while the per cent error is given in column 7.

In using this equation, it is necessary to determine the value of the two constants by means of at least two liquids of known viscosity. The samples used in this work were petroleum oils standardized by the Bureau of Standards. For the instrument with a ball of approximately 1¼ inch diameter, samples with viscosities of approximately 50 and 500 centipoise respectively would probably be most satisfactory. The importance of close temperature control for accurate measurements cannot be too strongly emphasized.

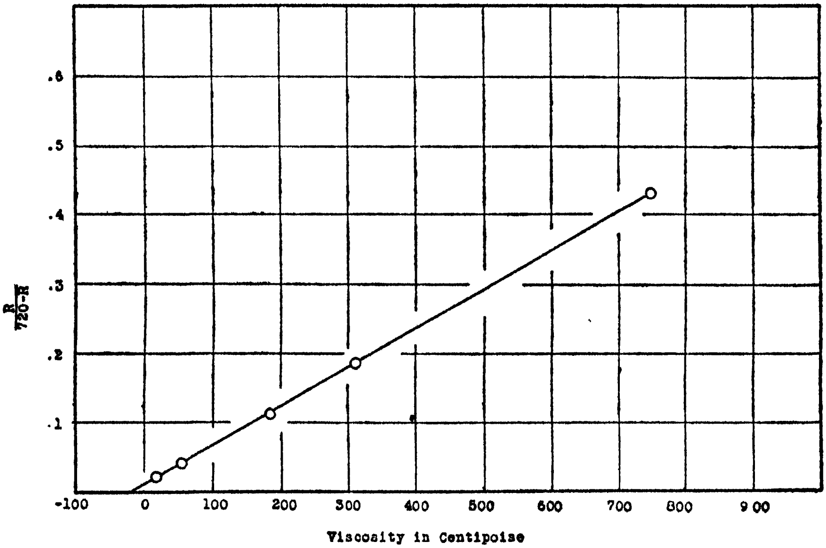


FIG. 2. GRAPHIC SOLUTION OF CONSTANT K

SUMMARY

An equation has been developed which makes possible the conversion of degrees retardation, a measure of viscosity determined by means of the torsion pendulum (Mojonnier-Doolittle) viscosimeter, into centipoise, the unit of absolute viscosity.

REFERENCES

(1) Coulomb: Experiences Destinées à déterminer la coherence des fluides et les lois de leur résistance dans les mouvements trés-lents. Memoires de L'Institute National des Sciences et Arts. Sciences Mathematiques et Physiques. Tomb Troisieme an IX, (1800).

(2) Babcock, S. M.: Report of Chemist. New York (Geneva) Agr. Expt. Station. 5th Annual Report. 316, (1886).

(3) Doolittle, O. S.: The torsion viscosimeter. Jour. Am. Chem. Soc., xv, 173, (1893).

(4) Doolittle, O. S.: Standardizing the torsion viscosimeter. Jour. Am. Chem. Soc., xv, 454, (1893).

(5) Mojonnier, T., and Troy, H. C.: The Technical Control of Dairy Products. Mojonnier Bros. Co., Chicago, Ill., 1st Edition 567, (1922).

EFFECT OF HEAT (OESTRUM) ON BUTTERFAT PERCENTAGE AND MILK YIELD*

LYNN COPELAND

Register of Merit Department, American Jersey Cattle Club, New York City

Most breeders of dairy cattle believe that the period of heat (oestrum) appreciably affects both the milk yield and butterfat percentage. This belief is usually based on individual observation of a limited number of cows. Unfortunately the actual literature on the subject is very limited.

McClandish (1) states that there is a decrease in milk production on the day of breeding and the day following. His report also shows an apparently compensating increase in milk flow during the two days prior to the period of oestrum. Hooper and Brown (2) found that most cows showed a decrease in both milk and fat yields during the day of most evident heat. A few cows, however, exhibited an increase in production while others showed no variation.

DATA

The last 2025 Register of Merit records completed and accepted by the American Jersey Cattle Club were used in the following study. In the making of these records approximately 23,000 supervisions were conducted. Testers are supposed to indicate on each report if a cow is in heat during the period of supervision. We believe that this was usually done and the reports containing these notations were studied. Not all of the reports showing heat periods were used however. Only those one-day reports were selected which were preceded and followed by apparently normal supervisions. The butterfat percentage of the supervision made during the heat period was then compared with the average percentage of the preceding and succeeding supervisions. Similarly the milk yields of the one-day test period conducted

---

* Received for publication July 18, 1929.

while the cows were in heat were compared with an average of the milk yields for the two days preceding the test and the two days following. A total of 211 such comparisons were made. The results were tabulated and are given in the accompanying frequency table (table 1).

TABLE 1

Frequency table showing variations in fat percentage and milk yield due to "heat"

AMOUNT OF VARIATION IN FAT PERCENTAGE	NUMBER OF VARIATES SHOWING INCREASE	NUMBER OF VARIATES SHOWING DECREASE	AMOUNT OF VARIATION IN MILK YIELD	NUMBER OF VARIATES SHOWING INCREASE	NUMBER OF VARIATES SHOWING DECREASE
per cent			*pounds*		
0–0 10	16	15	0– 0 5	28	30
0 11–0 20	19	18	0 51– 1.00	18	23
0 21–0.30	22	10	1.01– 1 50	12	21
0 31–0.40	12	10	1 51– 2 00	3	15
0 41–0 50	11	8	2.01– 2 50	8	11
0 51–0 60	13	8	2 51– 3.00	1	5
0 61–0 70	10	6	3.01– 3 50	3	3
0 71–0 80	4	2	3 51– 4 00	3	
0 81–0 90	5	1	4.01– 4.50	2	4
0 91–1 00	1	3	4 51– 5.00		4
1 01–1.10	5		5 01– 5.50		3
1.11–1.20	1		5 51– 6 00	2	1
1.21–1.30	1		6 01– 6.50	1	1
1 31–1.40	1	1	6.51– 7.00		2
1 41–1 50	1	1	7.01– 7 50		2
1.51–1 60	2		7 51– 8 00		
1.61–1.70	1		8 01– 8.50		
1 71–1 80			8 51– 9 00		
1.81–1 90	1		9.01– 9 50		
1.91–2 00	1		9 51–10.00		
2 01–2 10			Over 10.00		2
2.11–2 20					
2.21–2 30					
2.31–2.40					
2.41–2 50					
Over 2.50		1			

An examination of the data showed that in 75 cases the period of heat resulted in an increased milk flow while in 131 cases the yield was lessened. The average variation in milk quantity during the twenty-four-hour test periods conducted while the cows were in heat compared with the average yield of the two

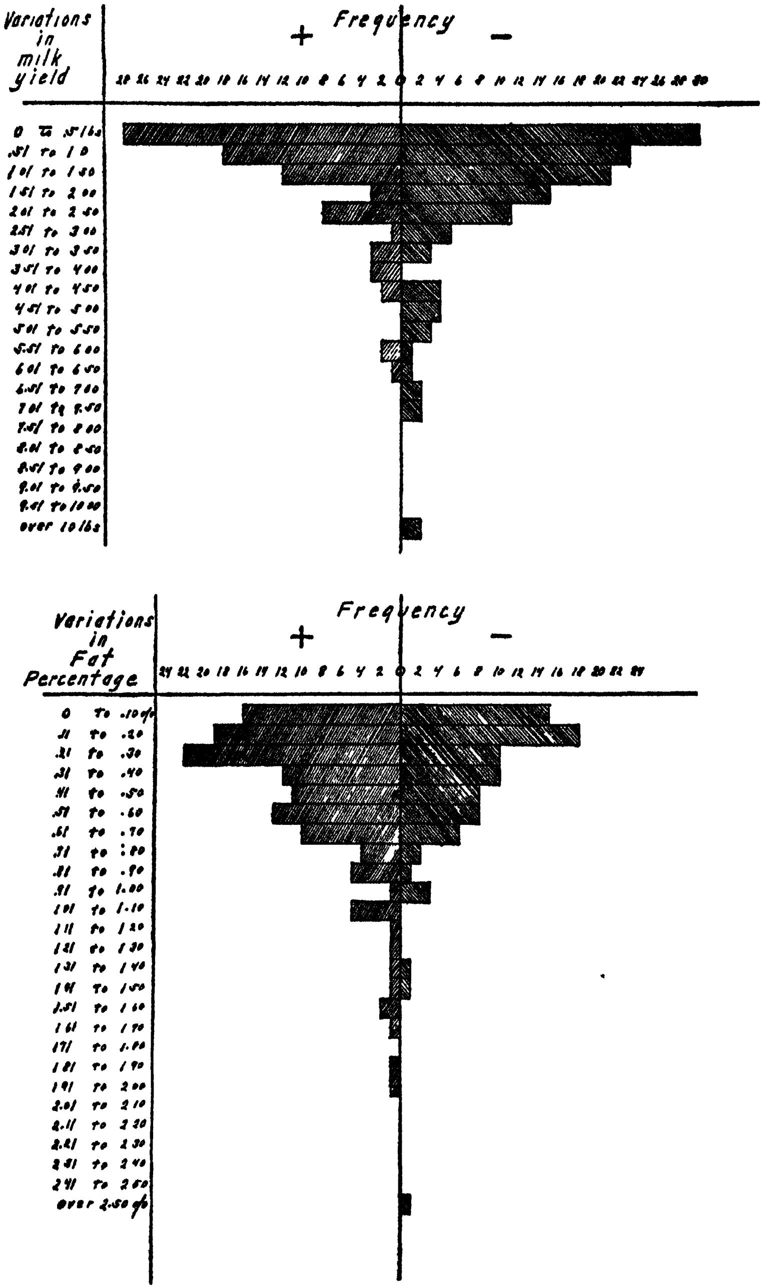


FIG. 1. VARIATIONS IN THE MILK YIELD AND FAT PERCENTAGE DUE TO "HEAT"

preceding and two succeeding days was 1.67 pounds The standard deviation in milk quantity was 2.354 ±0.077. For 153 cows or 75 per cent of the total number, the variation in milk production was less than 2 pounds. The total of all tests gave an average decrease in milk yield of 0.63 pound.

Butterfat percentage exhibited a slight increase during 126 of the 211 supervisions made during heat and in the remaining 81 supervisions the percentage declined. The average variation in butterfat percentage was 0.43 per cent and the average increase for all supervisions was 0.13 per cent. Standard deviation for butterfat percentage was 0.449 ±0.015.

The frequency table grouping the variates of butterfat percentage and milk yield variations into certain classes, together with the accompanying charts graphically picture the variation in milk yield and fat percentage attributable to heat. Since both of the graphs are somewhat skewed, milk yield toward the minus (decrease) side and fat percentage toward the plus (increase) side, it was thought that a definite correlation might exist between the two. However, the coefficient of correlation between milk yield and fat percentage during heat was only −0.1002 ±0.003. In 43 cases both milk yield and fat percentage increased during the heat period and in 58 cases they both declined. There were 73 times in which the milk yield declined slightly and the fat percentage increased a small amount, and in 27 cases the reverse was true. In the remaining 10 cases either butterfat percentage or milk yield remained constant, showing no variation.

Another comparison was made which is not shown in the table. The milk yields of the two days preceding each heat period was compared to the yield of the two days following heat. For 112 cows the milk flow of the two days just prior to the heat period exceeded the yield of the two days after heat and in the other 99 cases the opposite was true. In 136 instances the difference in the yields of these two periods amounted to less than 3 pounds.

SUMMARY

From the data presented it would appear that:

1. The effect of heat (oestrum) on milk yield and butterfat percentage is quite limited.

2. The slight difference exhibited seems with most cows to be toward a small decline in milk flow and an equally limited increase in butterfat percentage during the period of heat. Individual cows may show variation in the opposite direction or no variation at all.

3. No compensating increase could be noted in the milk production just prior to the heat period.

REFERENCES

(1) McClandish, A. C. 1926 Influence of the period of heat on milk production. Jour. Dairy Sci., ix, 67.
(2) Hooper, J. J., and Brown, P. E. 1919 Heat period and milk production. Breeders' Gazette, lxxv, 1215.

THE USE OF THE MOJONNIER MILK TESTER FOR THE ROUTINE DETERMINATION OF VANILLIN WITH PRELIMINARY DISCUSSION OF THE TOWT LEAD NUMBER*

L. V. TOWT

Research Laboratory of Standard Creameries Co., Ltd., Oakland, California

Most flavoring materials used in vanilla ice cream at the present time are either concentrated vanilla preparations or extracts which have been reinforced with vanillin. The choice of such materials over the straight vanilla extract has undoubtedly been brought about by the attempt of the ice cream manufacturer to prevent "freezing out," the desire of the consuming public for a stronger flavored ice cream, the advent of high solids ice cream which necessitates more flavor, and the regulations in certain states which prohibit the use of coumarin and other foreign flavoring materials.

Consequently, because vanillin is the most expensive ingredient of the extract and because it plays such an important part in the quality of the ice cream produced, its routine determination affords one of the most important means of checking the quality of the product purchased. Today most ice cream manufacturers buy in large quantities and contract for a year's supply in advance. They must feel assured that at all times during the period of the contract they are getting exactly what was originally specified.

For those dairy laboratories where both the Mojonnier and Babcock testers are found the following method affords a simple and very accurate means of determining the vanillin content and estimating the bean content of an extract.

PROCEDURE

Pipette 25 cc. of the extract into a petri dish cover and add 10 cc. of water. Evaporate to about 25 cc. under the suction fan

---

* Received for publication May 7, 1929.

on the hot plate of the Mojonnier Tester. The temperature of the fat hot plate may be kept at 60°C. during this process but care must be taken that the temperature does not rise above 60°C. Add 10 cc. water and evaporate again to about 25 cc.

Transfer the dealcoholized extract to an 18-gram cream test bottle which has previously been calibrated to 50 cc. To calibrate add exactly 50 cc. of distilled water at 20°C. and mark on the neck of the bottle. The transfer to the cream test bottle from the petri dish cover can be made very easily by using a glass funnel and stirring rod. Wash the petri dish cover clean using a wash bottle containing warm water.

Add 4 drops of Glacial Acetic acid and shake thoroughly. Add 10 cc. of standard lead acetate solution (80 grams C. P. crystallized lead acetate made up to 1 liter) make up to the 50 cc. mark with water and shake thoroughly. Cork the cream test bottle and centrifuge in a Babcock Centrifuge for five minutes at same speed used in making a fat test. Pipette off 25 cc. of the supernatant liquid, which should be clear, into a Mojonnier fat extraction flask and reserve the remainder for the estimation of the volume of lead acetate precipitate.

Extract the vanillin solution with three 20-cc. portions of ether which has previously been washed twice with distilled water, using the Mojonnier centrifuge after each shaking at the same speed and time as used in the fat test. Pour the ether extractions into a weighed solids dish and after the last extraction lower the dividing line by pipetting off the lower liquid. The same care used in pouring off the ether solution in the fat test should be used in this test.

Evaporate the ether solution of vanillin on the fat hot plate with the temperature not above 55°C. dry over sulphuric acid to constant weight and calculate the vanillin as grams per 100 cc. or as per cent by weight if the specific gravity of the original sample is known.

To check the accuracy of this method of determining vanillin different amounts of vanillin were added to an extract of known vanillin content and analyzed. The results are given in table 1.

The accuracy of this method even in samples containing large amounts of added vanillin is apparent from the table. In the official Hess and Prescott method, of which this is a modification, it is claimed that the vanillin extracted is never pure but contains varying amounts of gummy resinous matter; such is probably the case in this method, but apparently the error due to impurities just about offsets the loss of incomplete extraction.

TABLE 1

Accuracy of results secured by the proposed method of estimating vanillin

VANILLIN PRESENT IN EXTRACT	VANILLIN ADDED	TOTAL	FOUND	TOTAL RECOVERED	REMARKS
per cent	*per cent*	*per cent*	*per cent*	*per cent*	
0.40	0.2051	0 6051	0 635	105 0	Bourbon extract
0.40	0.8022	1.202	1.20	99.7	Bourbon extract
0 245	0.4797	0 7247	0.706	97.7	Tahiti extract
	2.02	2 02	2.02	100 0	Vanillin dissolved in 38 per cent alcohol
	0.491	0.491	0.53	108.0	Vanillin dissolved in 38 per cent alcohol
	1.43	1.43	1.42	99.3	Vanillin dissolved in 38 per cent alcohol
0 245	1 527	1 77	1.69	95.5	Tahiti extract
0.245	0.4797	0.7247	0.715	98.7	Tahiti extract
	0.6049	0.6049	0.582	96.4	Aqueous vanillin solution
0 245	0.4797	0.724	0.756	104.3	Tahiti extract
	1 381	1.381	1.396	101.0	Aqueous vanillin solution
	0.138	0.138	0.134	97.1	Aqueous vanillin solution
0.233	1.40	1.63	1.67	102.4	Bourbon and Tahiti 50–50, simple syrup added
0 294		0.294	0.294	100.0	Tahiti extract
0.147		0.147	0.150	102.0	Same as above diluted 1:1
Average..				100.5	

The determination of vanillin and the estimation of volume of lead acetate may be made easily in one day with this method, while the official methods would require at least three days. This method is also well adapted for volume as at least eight samples may be run at one time, and much more cleaner extraction can be made with the Mojonnier Flask than with a separatory funnel. The colorimetric method for determination of vanillin is shorter but involves the use of a colorimeter which is an instrument rarely found in a dairy laboratory and it does not allow for a lead number or bean matter estimation of the same weighed sample.

To determine the volume of lead acetate precipitate make up the remaining solution in the cream test bottle to the 45 per cent mark with water, cork, invert, and shake thoroughly, taking care that no particles of bean matter adhere to the bulb of the bottle. Centrifuge in the Babcock Centrifuge with the neck and corked end out at 1000 r.p.m. for five minutes. This is accomplished by inserting a round tube of wood about 6 inches long with a ⅝-inch diameter hole in the center in the bottle carrier on the centrifuge and placing the bottle neck in the hole in this cylinder of wood. This forces the lead acetate precipitate into the graduated neck where it can be measured with the dividers and read on the graduated scale.

A comparison of readings obtained in this manner was made with the normal lead number on six different samples with the following results:

The centrifuge used was a 36-bottle, 22-inch, steam driven, Babcock tester and was run at 1000 r.p.m. for five minutes.

SAMPLE NUMBER	LEAD NUMBER	READING ON 18-GRAM CREAM TEST BOTTLE
1	0.24	4.5
2	0.49	21.5
3	0.86	30.0
4	0.29	8 0
5	0.57	23.5
6	0.43	17.5

This simple test affords a very rapid method of checking the consistency of a product. It is particularly desirable when one buys on a "key sample" basis. In this case the seller submits a large sample of the flavoring material decided upon with the understanding that each shipment during the period of the contract shall conform to the composition of the key sample. After having carefully determined the lead reading of the key sample any variation of subsequent shipments can easily be detected without resorting to the tedious process of determining the official lead number.

A COMPARISON OF THE MODIFIED BABCOCK AND THE MOJONNIER METHODS FOR BUTTERFAT IN ICE CREAM*

E. S. CHASE AND F. G. KING

California Dairies Laboratory, Los Angeles, California

The object of this paper is to give the results of a comparison of the modified Babcock and the Mojonnier methods for butterfat in Ice Cream under factory conditions. The methods used in this paper were strictly factory methods used for standardization of the ice cream mix or for a check on this standardization. The Mojonnier method was used for standardization and [illegible] a Babcock test run on the same sample. The methods used are as follows:

MODIFIED BABCOCK

A 9-gram sample of the ice cream mix was weighed into the California standard 50 per cent 9-gram test bottle, using the standard Babcock torsion balance. Fifteen cubic centimeters of glacial acetic acid were then added to the bottle and agitated until all lumps were broken up. The acid used was commercial glacial acetic acid (98 to 99 per cent) and was kept in a tightly corked bottle. The sample was heated in a water bath to approximately 110°F. While still hot small portions of sulphuric acid were added and mixed with the sample. This was repeated until the sample developed a dark brown color (almost black) within two minutes standing. This is the most important part of the procedure and considerable experience was necessary to judge the proper color. The action was stopped by the addition of a few cubic centimeters of water and the sample centrifuged as usual. The standard five-, two- and one-minute centrifugings are satisfactory although a longer time is desirable on the last two. The samples were brought to 135°F. in a water bath and the fat read carefully (using glymol oil) with fine point dividers.

* Received for publication May 20, 1929.

The effect of further centrifuging was found to be very slight providing it was done properly in the first place. A 14-inch Burrell steam turbine was used at a speed of 900 to 1000 revolutions per minute. In a few cases a third washing and rapid centrifuging lowered the test about 0.05 per cent. The fat in this case was almost transparent.

An experienced operator can almost always obtain a perfect test with this procedure properly carried out. A test was considered perfect only if the fat column was a golden yellow, almost transparent, and homogeneous throughout. No burnt, charred or unburned substance was allowed in the fat column or in the liquid immediately below the column. Such a test shows improper manipulation and should be repeated. As a general rule an experienced operator can get a better looking fat column with this test than with the standard Babcock test for milk or cream.

The bottles used should be very carefully selected for accurate graduations. The graduations are to 0.5 per cent and are read to 0.10 per cent by judging tenths between the graduations. Duplicate tests showed a maximum variation of 0.1 per cent. An occasional 0.2 per cent or over was recorded but on further testing proved to be due either to faulty testing or a faulty bottle. The usual variation was well within the accuracy of reading, that is ±0.05 per cent.

It should be said, however, that this test is only applicable to ice cream that contains no fibrous flavoring material. Experiments show that strawberry, chocolate, and such flavors can not be tested satisfactorily by this method.

The Babcock-Gerber method as proposed by Moore and Morse[1] was tried in comparison with the modified Babcock and the Mojonnier. The results were not good, as all tests recorded were invariably from 0.2 to 0.4 per cent higher than either the Babcock or the Mojonnier. The amount of centrifuging affected the results considerably and the fat always carried the odor of butyl alcohol. The best results the authors gave for their method do not seem comparable to those we obtained. The only advantage noted was the ability to get a clear fat column with chocolate and strawberry ice creams but even here the results did not check with the Mojonnier figures.

---

[1] Moore and Morse: Jour. Dairy Sci., 1926, ix, 276–285.

MOJONNIER METHOD

The standard Mojonnier method for butterfat in ice cream was used.

TABLE 1

Comparison of Babcock and Mojonnier tests

BABCOCK TEST (DUPLICATES)		MOJONNIER TEST	DIFFERENCE, BABCOCK FROM MOJONNIER
per cent	*per cent*	*per cent*	*per cent*
11.1	11.1	10.94	+0.11
12.2	12.2	12.01	+0.19
11.0	11.0	10.94	+0.06
14.05	14.1	14.13	−0.05
11.20	11.20	11.15	+0.05
11.20	11.10	10.98	+0.17
11.0	11.10	10.96	−0.09
12.0	12.0	11.94	+0.06
12.0	12.0	12.00	+0.00
11.05	11.0	11.05	−0.03
11.2	11.1	11.14	+0.01
11.2	11.2	11.11	+0.09
18.1	18.1	18.00	+0.10
14.05	14.05	14.09	−0.04
18.5	18.6	17.96	+0.59
15.85	15.90	15.75	+0.12
17.20	17.20	17.11	+0.09
11.00	11.00	11.05	−0.05
13.94	14.00	13.95	−0.02
12.20	12.15	12.20	−0.03
Average 0.03 difference between duplicates		Average 0.10 difference from Mojonnier	

The fat determinations were all made at maximum speed, as they were used for factory standardization. The minimum time required for a fat and solids test was 40 minutes for 1 test, and 1 hour and 25 minutes for 4. The accuracy under these conditions was within ±0.05 per cent on duplicate samples. Curved five gram pipettes were used to weigh in the samples. In case of the slightest amount of rendered fat the sample was weighed directly into the bottle. Two extractions were used, a third not proving necessary.

RESULTS

The first three tables show the relative accuracy of the two methods and also the error on duplicate samples by the same method. Table 1 contains 20 Babcock and table 2 contains 20 Mojonnier duplicate determinations. Table 3 shows tests of 10 samples on which duplicates were run by both methods.

TABLE 2

Duplicate Mojonnier tests on separate samples

SAMPLE NUMBER	MOJONNIER TESTS ON SEPARATE SAMPLES		DIFFERENCE
	per cent	*per cent*	*per cent*
1	11.26	11.29	0.03
2	13.80	13.78	0.02
3	14.45	14.40	0.05
4	11.13	11.13	0.00
5	15.89	15.92	0.03
6	14.43	14.43	0.00
7	11.41	11.46	0.05
8	11.04	10.93	0.11
9	14.44	14.37	0.07
10	12.63	12.59	0.04
11	11.35	11.35	0.00
12	11.13	11.08	0.05
13	11.15	11.15	0.00
14	17.51	17.55	0.04
15	11.96	12.01	0.05
16	11.10	11.12	0.02
17	10.92	10.91	0.01
18	11.06	11.02	0.04
19	11.14	11.14	0.00
20	16.02	15.98	0.04

Average variation 0.03 per cent

Seven hundred and fifty-four tests were recorded. The Mojonnier test was in all cases used as the standard. The Babcock tests are divided into three classes; those that yield higher values, those that check exactly, and those that yield lower values than the Mojonnier method. The results are considered to 0.01 per cent butterfat.

273 tests were lower than Mojonnier
31 tests were the same as Mojonnier
450 tests were higher than Mojonnier

TABLE 3

Comparison of duplicate tests by both methods

SAMPLE NUMBER	BABCOCK TEST		MOJONNIER TEST		DIFFERENCE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	17.70	17.70	17.78	17.78	−0.08
2	13.70	13.65	13.56	13.63	+0.08
3	10.50	10.50	10.28	10.28	+0.22
4	10.70	10.65	10.59	10.59	+0.08
5	10.60	10.60	10.59	10.61	−0.00
6	10.50	10.50	10.36	10.42	+0.11
7	10.70	10.70	10.71	10.69	0.00
8	12.00	12.00	11.97	11.95	+0.04
9	10.50	10.45	10.49	10.41	+0.03
10	12.00	12.00	11.98	12.01	0.00

TABLE 4

Variation of Babcock and Mojonnier tests

GROUP NUMBER	NUMBER OF TESTS	PERCENTAGE OF TOTAL TESTS	RANGE OF VARIATION FROM STANDARD MOJONNIER TESTS
			per cent
1	5	0.6	±0.5 to 0.6
2	8	1.1	±0.4 to 0.5
3	21	2.8	±0.3 to 0.4
4	72	9.5	±0.2 to 0.3
5	204	28.8	±0.1 to 0.2
6	444	59.2	±0.0 to 0.1
Total............	754	100.0	

The tests are grouped as to their variations from the Mojonnier test in table 4. An average of all the tests shows that the Babcock method gave results which are 0.04 per cent higher than the Mojonnier method. A further check of the data shows 45.2 per cent of the tests check within ±0.06 per cent, that is within Mojonnier accuracy.

All samples in groups one to three inclusive were retested to check any possible errors in testing. Any mistakes found were further rectified by a third test.

DISCUSSION

This paper to the best of our knowledge represents a more comprehensive study than has heretofore been published on the relative accuracy of the Mojonnier and Modified Babcock Methods when applied to ice cream. It represents tests, all of which were made under strictly factory conditions. About 85 per cent of the Babcock tests were made by one man and about 90 per cent of the Mojonnier tests by another operator. The other tests were interchanged between the two men. Different operators were used on both tests to remove the personal element as much as possible. No appreciable difference could be noted with either operator on either test.

Eighty-six per cent of the tests were satisfactory if we consider ±0.2 per cent as sufficient accuracy. The other 14 per cent are of doubtful use in standardization. No explanation is offered for these tests nor is any remedy proposed. A correction of 0.04 per cent subtracted from all Babcock tests would make a greater number of tests check with the Mojonnier method. The reason for this is that there are 450 tests higher and only 270 tests lower.

An attempt to analyze the fat column of a Babcock test by the Mojonnier method gave no conclusive results. Determinations on half a dozen samples showed that the fat is never 100 per cent pure. The results did not show consistently that the fat column on a sample testing high by the Babcock method, actually contained a smaller percentage of ether soluble material than those samples in which the agreement was better although there was some indication of it. The fat columns obtained in the Babcock determinations tested from 97.53 to 98.7 per cent fat and the balance was moisture. This was not checked very thoroughly but it is logical to believe that a sample that tests high by the Babcock method would yield a fat column which contains a lower percentage of fat. A low test could be explained by incomplete separation of the fat from the acid liquid.

The question has been raised whether it is justifiable to read a cream test bottle to 0.1 per cent. The theory is that it is false accuracy to read closer than the smallest division on the graduated scale. We have assumed nevertheless that it is possible, and our results have confirmed it. However, this is only possible by using the very best equipment and the most careful technique. It must be said to the credit of our glassware manufacturers that the test bottles supplied are very accurate, to a much greater degree of precision than is required by law.

Another question is of the relative merit of using a 9-gram sample in a milk bottle and doubling the result, as against this method. According to theory this should be the best as no "false accuracy" is involved in reading to 0.2 per cent. However, on doubling a test a meniscus error is introduced which might offset any greater accuracy in reading. By comparison in this laboratory the milk bottle invariably showed from 0.2 to 0.6 per cent higher than the corresponding cream bottle test, which test has been shown to average slightly higher than the Mojonnier. The conclusion was that the cream bottle test using glymol to remove the meniscus gave the most consistent results. However, there are many other small variations in this test, such as the strength of the acetic acid used, strength of the sulphuric used and relative amounts of these reagents, that were only worked out so as to give satisfactory tests without regard to their effect, if any, on the test. There is still room for much research on these and many other problems of this test.

CONCLUSIONS

Forty-five per cent of the modified Babcock tests checked with the Mojonnier and 86 per cent were satisfactory, that is within ±0.2 per cent of the Mojonnier test. No reason can be given as to why some samples do not check within ±0.2 per cent. The maximum variation was approximately 0.5 per cent. The Babcock tests averaged 0.04 per cent higher than the Mojonnier tests.

ACKNOWLEDGMENT

We are indebted to the Division of Dairy Industry of the University of California, particularly to Dr. C. L. Roadhouse and D. H. Nelson, whose comments and criticisms on this paper are gratefully acknowledged.

VARIATIONS IN THE MILK FROM DIFFERENT QUARTERS OF THE SAME UDDER: THEIR SIGNIFICANCE IN STUDIES OF COAGULABILITY*

ANNE G. BENTON

Research Laboratories, Bureau of Dairy Industry, U. S. Department of Agriculture

The study of the factors determining the coagulability of milk by heat is complicated by a number of possibly interacting variables difficult to control. Moreover, as has been indicated elsewhere[1] each sample is an individual system, so that milk from one normal cow can not be used as a check on that from another, neither can a sample from one milking be used as a control on a previous or subsequent milking from the same cow. Variations in the thermostability, the alcohol test, and the hydrogen-ion concentration of herd milk have been attributed, and may be due, to changes in feed or environmental conditions. It is well known that milk at the beginning or end of lactation is unstable and there is a good deal of evidence that subacute infectious processes in the udder may cause alterations in the balance of ingredients without noticeably reducing the volume of milk secreted.

In studying the day to day variations in the milk of individual cows, it was noticed that not only does the milk of a single cow vary from one milking to another but that it varies even from different quarters of the udder. It was found that if quarters are milked separately the samples may be no more alike than if they had been obtained from different cows. The four quarters of the udder, therefore, are separate units physiologically as they are anatomically. Furthermore these variations are not constant—samples from each quarter vary from day to day just as does the total pooled sample. The data in table 1, obtained from six morning milkings over a period of two weeks, are illustrative.

---

* Received for publication May 22, 1929.

[1] Benton, A. G., and Albery, H. G.: Jour. Biol. Chem., lxviii, 251 (1926).

The hydrogen-ion concentrations were determined electrometrically. The coagulation times were determined after the milk had been forewarmed and condensed to 18 per cent total solids.

TABLE 1

Differences in milk from different quarters of the udder of cow 258 of the Beltsville herd

DATE	QUARTER OF UDDER	VOLUME	FAT	SERUM SOLIDS	pH	ALCOHOL TEST	COAGULATION AT 120°C.	BACTERIAL COUNT
		cc.	*per cent*	*per cent*			*minutes*	
July 20	Left rear		1.7	8.52	6.65	–	9	None taken
	Left fore		2.0	7.9	6.78	–	15	None taken
	Right fore		2.1	7.76	6 61	+	7	None taken
	Right rear		2.0	8.1	6.62	–	13	None taken
July 22	Left rear	1,700	3.0	8.52	6.62	–	9	19,000
	Left fore	1,460	3.0	8.55	6 63	–	8	16,900
	Right fore	1,150	3.0	8.56	6.62	–	6	12,500
	Right rear	2,000	3.0	8 59	6.62	–	12	15,100
July 24	Left rear	1,770	2.5	9.23	6 55	–	19	2,000
	Left fore	1,140	2.1	8.01	6.66	–	17	69,000
	Right fore	1,600	2.3	8.45	6.58	–	8	1,900
	Right rear	1,960	2.4	8.71	6.65	–	5	1,300
July 27	Left rear	1,470	2 9	8.7	6.67	+	3	14,300
	Left fore	1,030	2 3	8.34	6.77	–	5	10,200
	Right fore	1,445	2.7	8.60	6.67	+	0*	4,700
	Right rear	1,540	3.0	8.72	6.65	+	4	12,800
July 29	Left rear	1,575	2.6	8.84	6.66	+	5	None taken
	Left fore	1,000	2.5	8.31	6.84	–	12	None taken
	Right fore	1,455	2 4	8.5	6.70	+	3	None taken
	Right rear	1,555	2 5	8.73	6.63	+	5	None taken
August 3	Left rear	1,630	2.9	8.46	6 66	–	10	None taken
	Left fore	1,120	3.2	8.14	6.75	–	16	None taken
	Right fore	1,490	3.3	8.41	6.65	+	6	None taken
	Right rear	1,580	3.0	8.3	6.65	+	8	None taken

* Coagulated before it reached 120°C.

Variations similar to these in kind and degree were observed in a number of cows in the Beltsville herd of the Bureau of Dairy Industry, indicating that such cows are fairly common. Indeed, it may be the general rule rather than the exceptional state of affairs. In the work done so far, alcohol tests were made in the stable at milking time and cows which gave alcohol positive milk from one quarter and negative from another were chosen for further study. It should be noted that in the case here presented all four samples were alcohol negative on July 24, and yet they were alike in no other particular.

Such variations when encountered in a cow giving several liters at a milking provide an excellent opportunity to study certain factors affecting stability, since obviously other variables such as age of cow, stage of lactation, change of feed, digestive peculiarities or upsets, weather, sunlight, and exercise are completely ruled out. All four samples are elaborated simultaneously from an identical blood supply, and the difference in ingredients, hydrogen-ion concentration and coagulability must be due to differences in the secreting mammary epithelium or to chemical changes that take place while the milk is still in the udder. Studies of the variations in type and numbers of the udder bacteria should yield significant results. In the observations made thus far high counts were not so apt to accompany loss of stability as to precede it. This would point to the effect of the bacteria on the secreting cells rather than on the milk itself, but not enough work has been done to justify any definite opinion.

As the writer is no longer able to continue this work these suggestions are offered to other investigators for what they may be worth.

SEASONAL VARIATIONS IN THE FREEZING POINT OF MILK*

J. H. BUCHANAN AND O. E. LOWMAN

Department of Chemistry, Iowa State College, Ames, Iowa

It has been reported by a large number of workers that the freezing point of milk was quite definite. On reflection that is quite a reasonable assumption because the compounds of milk, sugar and ash, which would exert the greatest effect in lowering the freezing point, are the ones that are known to be the least variable in milk. An examination of the work of a large number of investigators in this field will show that there is an agreement with regards to the fact that the freezing point of normal milk falls within rather narrow limits. The highest freezing point, −0.540°C., is reported by Gooren (1) and the lowest limit, −0.575°C., by Ducrose and Imbert (2). Our investigations show the extreme limits to be between −0.537 and −0.582°C. (table 1).

The effect of the season on the depression of the freezing point has received very little attention. Stoecklin (3) after examining 2500 samples makes the following generalization: milk, freshly drawn, from any cow, whatever the breed, from whatever region of the country, from animals in stable or in pasture, whether poorly or substantially fed, whether drawn from a period near or remote from parturition, in winter or summer, morning or evening, whether yield be scant or abundant, has a definite freezing point which varies but little from −0.550°C. although under various influences the chemical composition changes in enormous proportions. Hortvet in his very extended work on the cryoscopic index of milk (4) seems not to have noticed the seasonal variation. However, the authors (5) noticed the effect of the season on the depression of the freezing point but sufficient data was lacking at that time. Since then a larger amount of data have been collected. All determinations were made on mixed,

---

* Received for publication June 4, 1929.

unpasteurized, herd milk using the Hortvet Cryoscope. The freezing was carried out immediately after obtaining the sample.

EXPERIMENTAL DATA

Table 1 for the most part is self-explanatory except the wide variations in the same month, and even from day to day, are difficult of explanation. Probably an explanation on meteorological grounds holds possibilities. Jackson and Rothera (6) were able to show some correlation between weather and conductivity of milk serum. On hot, dry days the conductivity was high while on cold days with high humidity the conductivity was low.

TABLE 1

Distribution of freezing points of herd milk (by months, 1924–1927 inclusive)

(Figures in months column indicate number of determinations)

FREEZING POINT DEPRESSION	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	TOTAL
°C.													
0.5800–0.5824		1				2							3
0.5775–0 5799	2						1	2					5
0.5750–0.5774		1											1
0 5725–0 5749													0
0 5700–0.5724		1					1	1				1	4
0.5675–0 5699	3	1	1			2	2	2				2	13
0 5650–0 5674	2	1	1	1				1			1		7
0 5625–0 5649		3		1		1	3	3		1	1		13
0.5600–0 5624		2	1	3	5	2	3	4		1			21
0 5575–0 5599	2	1	2	2	2	2	3	1			1		16
0 5550–0 5574			1	1	3	4	4	2		3			18
0.5525–0.5549			1	2	1	4	3						11
0 5500–0 5524			1	1	2	3	1				1		9
0 5475–0 5499			2		2	1							5
0.5450–0.5474				1									1
0.5425–0.5449					3	1							4
0.5400–0.5424				1									1
0.5375–0.5399													0
0.5350–0 5374										1			1

Their analyses for lactose content showed higher values when the conductivity was low and vice versa. However, our attempt at correlation of meteorological data and the wide variations was not very successful.

The data for October, November and December (1927) should be mentioned as it was collected during one season only. This season was rather exceptional as July, August and first part of September were very dry resulting in very poor pasturage conditions. This was followed by much rain and warm weather practically duplicating pasturage conditions found in early spring.

TABLE 2

Average freezing point depression (by months and years)

MONTHS	1925	1926	1927	GENERAL AVERAGES
January	−0 5673		−0.5627	−0.5650
February	−0.5672		−0.5622	−0.5647
March	−0.5636	−0.5587	−0.5517	−0.5580
April	−0.5572	−0.5548	−0.5581	−0.5567
May	−0.5566	−0 5536	−0 5577	−0.5559
June	−0.5556	−0.5645	−0.5593	−0.5598
July	−0.5567	−0.5633	−0.5561	−0.5587
August	−0.5604	−0.5626	−0.5635	−0.5622
September				
October			−0.5549	
November			−0.5594	
December			−0 5692	

The data for the other months represent normal conditions found in this climate.

Attention is called to the fact that in the last column of this table, the greater number of depressions fall between the limits of −0.5475 and 0.5724°C.

Table 2 was constructed from the data shown in table 1 representing the freezing point depressions between the limits of −0.5724 and −0.5475°C. defined by the heavy horizontal lines. It was thought that the other data are too much scattered and would greatly magnify the averages. The data in this table represents an average of the freezing point depressions per month during the years over which the investigation was conducted. The last column represents an average by months during the entire period.

The curves in figure 1 were constructed from the data in table 2.

Curve A (1925). This curve, as well as B and C, was made from the average freezing point depressions from the milk of a mixed herd. During the months of January and February the herd received the usual winter rations of silage and rough dry feed. However, in March a small amount of pasturage became available, with a luxuriant growth in April, May, and June with a waning in July to a scant growth in August due to the hot, dry weather.

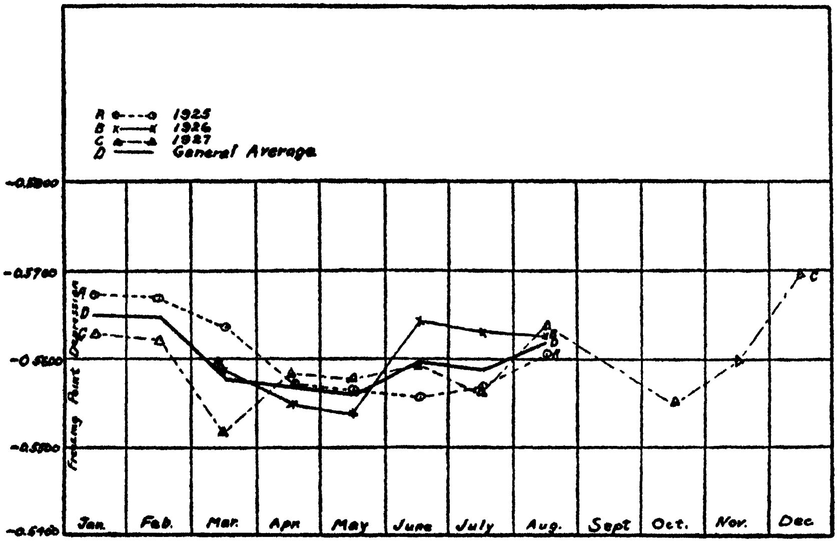


FIG. 1. EFFECT OF SEASON ON DEPRESSION OF FREEZING POINT

Curve B (1926). Normal spring pasturage conditions existed for the spring months (March, April, and May). Exceptional drouth conditions prevailed during June, July, and August, resulting in very scant pasturage especially during June. A consequent lowering of the freezing point depressions resulted.

Curve C (*1927*). Unusual pasturage conditions existed during March. Average conditions prevailed during remainder of the spring months with the usual drouth conditions during August. However, warm September rains resulted in a practical duplication of spring pasturage with an attendant fluctuation of the freezing point depression.

Curve D (*general average*). This curve was constructed from a general average of the freezing point depressions of the other curves. It is carried only to September 1 as the work through October, November, and December was conducted only one season (1927). The pasturage conditions during October and November were exceptional rather than usual.

DISCUSSION OF DATA

Jackson and Rothera, University of Melbourne (6), were able to show that hot dry weather increased the electrical conductivity of raw milk while cold wet weather diminished it. Also, they were able to show that when the conductivity was high the lactose content was low and vice versa. The variation in conductivity would indicate a variation in the soluble, ionizable mineral content of the milk. Since a balance is maintained between this mineral content and the lactose content, the greatest factors causing the freezing point depression, this would account for the rather narrow range of the freezing point depression of milk. However, since this is thought to be dependent on meteorological conditions it may be one of the principal factors in the wide variations from day to day but it does not account satisfactorily for the general seasonal variation.

Cranfield, Griffiths, and Ling (7) were able to show a considerable variation in soluble ash content of milk with the months of the year, the percentage being much higher in summer than in winter. Sodium chloride is conceded to be the principal constituent in the soluble ash of milk and one of the most variable factors, hence it is reasonable to assume that it is the factor used to maintain the osmotic equilibrium between the blood and milk secretion in the animal.

Woodman, Blunt, and Stewart (8) were able to show that the mineral content of pasturage, especially the calcium content, showed a very definite seasonal variation, increasing to a maximum in the dry mid-season and later falling off again. This increased calcium content if transferred to the milk would not greatly affect the freezing point depresssion as possibly two-thirds of the total phosphate is in suspension as $Ca_3(PO_4)_2$. These investigators have shown that the calcium content varies from season to season (9). They are inclined to believe that meteorological conditions produce these variations. Fagan (10) has been able to show that when grasses, cut or grazed very short, are much more leafy when they grow out again and consequently the chemical composition changes greatly. This is undoubtedly what happens when the Autumn rains come on. However, this worker has shown that this type of growth is much poorer in nutritive value than new spring growth.

Undoubtedly, when cattle are first placed on new pasture in the spring a physiological disturbance results due to the nutritive content of the grass and meteorological changes. This brings on a disturbance of the osmotic equilibrium maintained in the body and consequently a variation in the freezing depression of milk results. Curve C, figure 1, would justify these conclusions since practical spring pasturage conditions were duplicated in October and hence the raising of the freezing point depression.

SUMMARY

1. There is a distinct seasonal variation in the freezing point depression of milk; the greater depression being in the spring months, the lower portion during the winter months.

2. Change in meteorological conditions offer an explanation of the wide variations of the freezing points encountered from day to day.

3. The seasonal variation of the freezing point depression is due to a change in the osmotic equilibrium maintained in the body of the animal. While meteorological changes may be a factor, the change in chemical content of the animal's food during the season is the controlling factor in the seasonal variation of the freezing point.

REFERENCES

(1) Gooren: Centr. Bakt., Parasitenk., xxxv, 641.
(2) Ducrose and Imbert: Bull. Sci. pharmacol., vii, 65.
(3) Stoecklin: Ann. fals., iv, 234.
(4) Hortvet, J.: Jour. Ind. and Eng. Chem., 1921, xiii, 198.
(5) Buchanan, J. H., and Lowman, O. E.: Jour. Dairy Sci., 1926, ix, 192.
(6) Jackson, L. C., and Rothera, A. C. H.: Biochem. Jour., 1914, viii, 1.
(7) Cranfield, Griffiths, and Ling: Jour. Agr. Sci., 1927, xvii, 87.
(8) Woodman, Blunt and Stewart: Jour. Agri. Sci., 1926, xvi, 205.
(9) Woodman, Blunt and Stewart: Jour. Agri. Sci., 1927, xvii, 209.
(10) Fagan: Univ. College Wales, Aberystwyth. Agri. Dept. Advisory Bull., 1927, ii, 23.

THE OVARIAN HORMONE CONTENT OF PREGNANT COW'S URINE*

C. W. NIBLER AND CHAS. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia, Missouri

INTRODUCTION

Because of the close correlation between the mammary glands and the reproductive organs, the pioneer investigations of Allen and Doisy (2, 3, 4, 6, 9), Frank and associates (12, 13, 14), Parkes and Bellerby (24) and others, concerned with an ovarian hormone, are of especial interest for they have furnished fundamental knowledge concerning the hormone which is believed to activate or stimulate the growth of the mammary gland.

That the ovaries are the organs which cause the phenomenon of "heat" in females has long been known. It was shown by Knauer (18) that this phenomenon could be abolished when both ovaries were removed.

Similarly it was found by Halban (15) that by the removal of the ovaries from guinea pigs the uterus and mammary gland ceased to develop; however, when ovarian grafts were made, the generative organs developed normally.

Although the early work proved that the ovaries controlled the functions of the generative organs, a method was lacking by which specific substances from the ovaries could be isolated and tested.

The pioneer investigations leading to a definite test of the presence and potency of the oestrous producing hormone was made by Papanicolaou and Stockard (22) who upon microscopical examination of the vaginal contents of the guinea pig showed that there were distinct changes in the cellular content of the uterus and vagina during the oestrous cycle.

---

* Received for publication June 17, 1929. The data presented in this paper formed part of a thesis presented by C. W. Nibler in partial fulfillment of the requirements for the Degree of Master of Arts in the Graduate School of the University of Missouri, 1929.

Later the oestrous cycle was studied by means of the vaginal smear technique in the rat by Long and Evans (21) and in the mouse by Allen (1).

The early work of Halban (15) and of Knauer (18) proved rather conclusively that a secretion from the ovaries carried by the blood stream served to control the sexual functions. Therefore, it could be supposed that ovarian control was probably due to some hormone elaborated by the ovaries. The first to show that there was a specific hormone secreted by the ovaries were Allen and Doisy (3) who produced typical oestrous changes, as shown by vaginal smears, in spayed animals, by the injection of liquor folliculi from hog ovaries. The discovery of the oestrous producing hormone in the follicular fluid soon led to its isolation from other sources as shown in table 1.

TABLE 1

Source of an ovarian hormone as tested for by vaginal smear technique

	INVESTIGATOR
Ovaries (sheep)	Allen and Doisy (1923)
Liquor folliculi	Allen and Doisy (1923)
Corpus luteum (Human)	Allen, Pratt, and Doisy (1925)
Wall of corpus luteum	Zondek and Ascheim (1925) (26)
Placenta	Allen and Doisy (1924)
Maternal placenta	Doisy, Ralls, Allen, and Johnston (1924)
Fetal placenta	Parkes (1928)
Amniotic fluid	Parkes (1928)
Blood of females (during oestrous cycle)	Frank, R. T., Frank, M. L., Gustavson, R. G., and Weyerts, W. W. (1925)
Blood of pregnant females	Fels (1926)
Urine (during oestrous cycle)	Loewe (1926) (20)
Urine (during pregnancy)	Veler and Doisy (1928)

QUANTITATIVE TEST OF THE HORMONE

After the isolation of the oestrous producing hormone there was need of a method of measuring, in a comparative manner, the potency of extracts of the hormone.

The *rat unit* devised by Allen and Doisy (4) is defined as follows: "The quantity of material necessary to induce oestrous as judged by the smear method in an ovariectomized sexually mature rat weighing 140 ±20 grams." Allen and Doisy further state, "That for physiological reasons we generally make three subcutaneous injections at intervals of four hours. The rat unit then is the minimum amount so injected which produced full oestrous growth in the genital tract. Although we are convinced that this method of testing is roughly quantitative, we have not yet made a careful study of its limitations."

Although the rat unit is recognized as having certain limitations as shown by Allen and collaborators (4), Coward and Burns (8), Evans and Burr (10), and Bugbee and Simond (7) it affords a fairly accurate quantitative test of the potency of the extracts studied.

For other information concerning the ovarian hormone the reader is referred to the reviews by Allen and Doisy (5) and Parkes (23).

OBJECT OF THE INVESTIGATION

The early work clearly showed that there is a close relation between the reproductive organs and the mammary gland. The exact cause of the growth of the mammary glands, however, has been a subject of great speculation. All the parts of the gonads, the placenta, and the fetus have been suggested as the source of the hormone which stimulates the growth of the mammary gland during pregnancy. Upon the isolation of the oestrous producing hormone and the development of the rat unit test the question immediately arose as to the effect of this hormone on the growth of the mammary gland.

There are two methods of attack of the problem. One method consists in injecting definite amounts of the hormone into immature gonadectomized experimental animals and observe the extent of the growth of the mammary gland and the initiation of milk secretion. It is interesting to note that experiments of this type are beginning to be reported which would indicate that the oestrous producing hormone is capable of producing some growth of the gland. In preliminary work, we have been able to induce considerable growth of the gland in castrated male rabbits. A more extensive report of this work will be made later.

A second method of study of the influence of the oestrous producing hormone on the normal development of the mammary gland and the initiation of milk secretion consists in correlating the amount of the ovarian hormone present in the blood (Fels (11)) during various stages of pregnancy with the growth of the gland and the yield of a secretion.

Preliminary studies with blood were unsatisfactory due to the amount of blood required. Upon the discovery of Veler and Doisy (25) that the hormone in the blood of pregnant women was being excreted in the urine, a simple method was available by which to study the changes in the concentration of the hormone in the blood and which is excreted in the urine during pregnancy when the growth of the gland is most rapid. That the hormone is also excreted in the urine of pregnant cows was immediately demonstrated in a few preliminary tests. Recently Hisaw and Myers (17) report the presence of the hormone in the urine of a limited number of pregnant cows.

The object of the present paper is to report the results of an extensive series of tests of urine of cows in all stages of pregnancy and to show that the increase in the concentration of the hormone parallels the growth of the mammary gland of first calf heifers.

PROCEDURE

The urine for this investigation was collected from cows of the Holstein, Jersey, Guernsey, and Ayrshire breeds of the Missouri Station dairy herd. At the beginning it was necessary to watch the cows continuously to obtain samples of urine for a definite period. Later a method was discovered whereby urination was affected so that collections were made at two hour intervals.

The method consisted of a gentle massage, starting below the ventral commissure of the vulva and taking its course upward and laterally terminating beside the labium vulva (see fig. 1). After a short period of stimulation urination was produced.

While there have been published complicated methods of extracting the ovarian hormone from urine, Doisy (unpublished

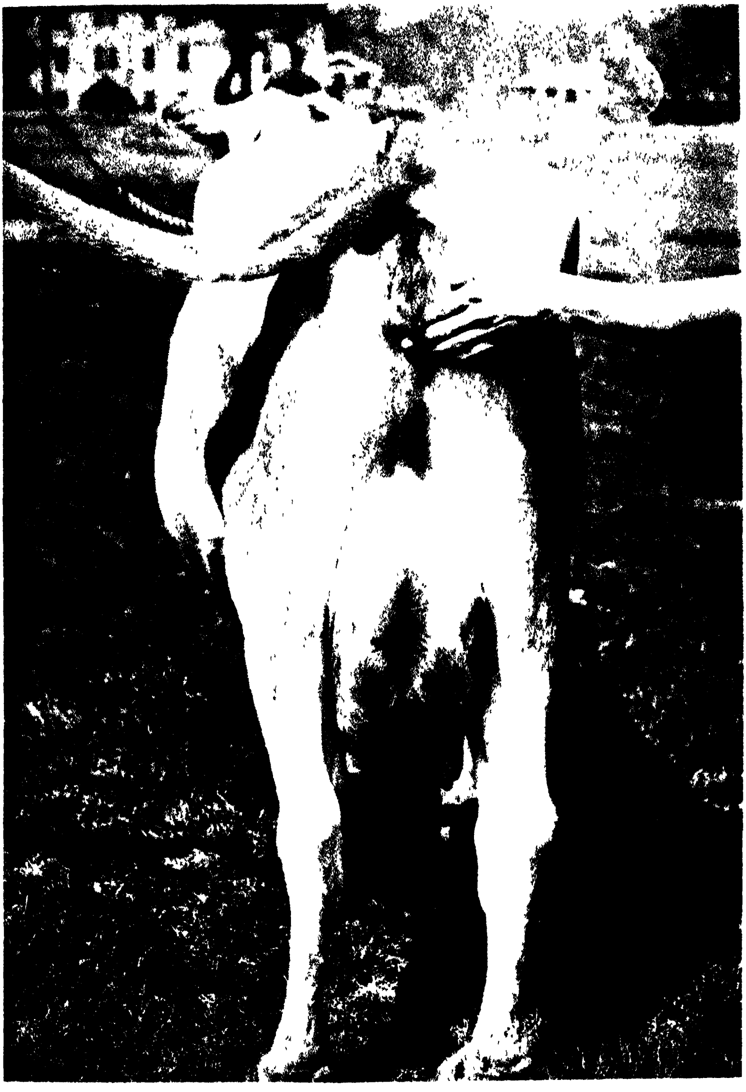


FIG. 1. THE POSITION OF THE HAND BELOW THE VENTRAL COMMISSURE OF THE VULVA, WHERE IT IS PLACED AT THE START OF THE MASSAGE WHICH CAUSES THE COW TO URINATE

From this position the hand takes its course upward and laterally terminating beside the labium vulva.

work) has devised an extremely simple procedure which was followed in all of our work. It was found that oil (olive oil was used) has an affinity for the hormone when mixed with the urine.

The method employed was to add 50 cubic centimeters of oil to 2 liters of urine or a proportional amount of oil to larger or smaller volumes of urine. After the addition of the oil, the sample was mixed for one hour and then the mixture was placed in a separatory funnel until the urine and oil had formed separate layers. The urine was then drained off and discarded while the oily residue was drained into test tubes. The oily residue was cleared in a centrifuge, pipetted off, and placed in a clean stoppered test tube for a later quantitative determination.

Allen and Doisy rat unit test was used in determining the potency of the extracts. At the time of the first injection a vaginal smear was obtained as a check to definitely show that the animal was in the dioestrous stage. Vaginal smears were obtained from the injected rats forty-eight and fifty-six hours after the time of injection. These slides were then fixed by means of a flame and stained in Delafield's hematoxylin and eosin.

Slides upon microscopic examination showing cornified cells were designated as positive. The minimum amount of the hormone to produce this condition was considered one rat unit. Slides showing a mixture of leucocytes and epithelial cells were designated as a plus minus reaction. This type of smear suggests a small amount of development but not enough to be positive. The predominance of leucocytes and to a less extent nucleated epithelial cells were designated as a minus.

PRESENTATION OF DATA

Because of the lack of information as to the efficiency of the method of extraction, considerable preliminary work was necessary. It was found that when 10, 15, or 20 cc. of oil were added to two liters of urine practically the same number of rat units were extracted. Similarly, the length of time of stirring apparently had little effect on the efficiency of the absorption of the hormone by the oil for a stirring period of thirty minutes gave results identical with the 120-minute period.

The efficiency of the method of extracting the hormone was further tested by adding known amounts of the hormone to urine and then extracting and testing each sample. It was found that the number of rat units extracted was practically identical with the number added to the urine. The results are shown in figure 2. These data indicate further that the oil is equally efficient in removing the hormone whether in small or large concentration.

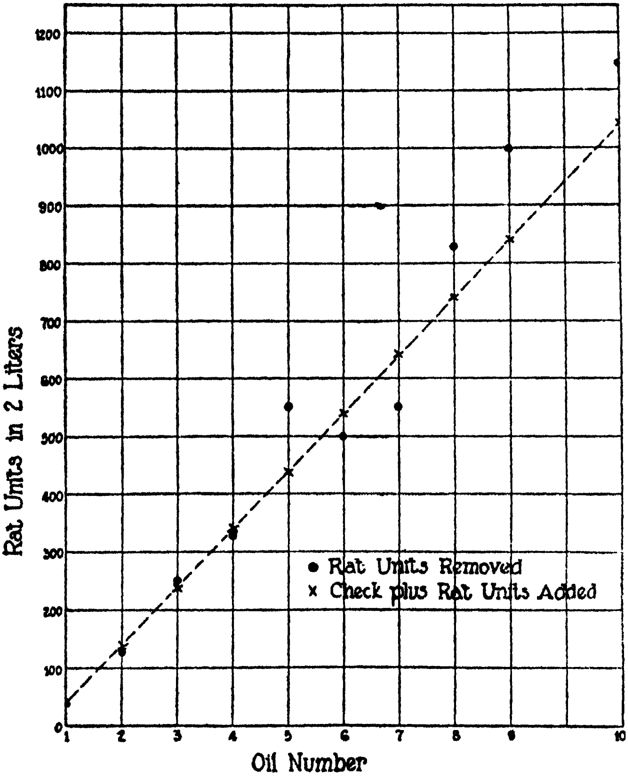


FIG. 2. THE EFFICIENCY OF THE METHOD OF EXTRACTION USED WITH INCREASING AMOUNTS OF THE OVARIAN HORMONE

The broken line shows the amount of the ovarian hormone in the urine plus the number of rat units added; the dots indicate the number of rat units recovered. These results indicate that the ovarian hormone in varying concentrations is removed by oil with equal efficiency.

The length of the period of urine collection necessary to obtain a representative sample of the excretion of the hormone was determined by comparing the excretion by twelve-hour and twenty-four-hour periods.

TABLE 2

Rat units in cows urine at various stages of gestation

COW NUMBER	BREED	AGE		STAGE OF GESTATION	AVERAGE URINE, 24 HOURS	AVERAGE RAT UNITS, 24 HOURS	AVERAGE RAT UNITS PER LITER
		yrs.	*mos.*	*days*	*cc.*		
426	Guernsey	3	4	281	6,235	818	131
190	Jersey	3	6	281	6,605	629	95
557	Holstein	3	5	273	8,154	782	95
426	Guernsey	3	4	271	5,504	799	145
193	Jersey	3	4	270	9,450	872	92
190	Jersey	3	6	268	9,400	462	48
557	Holstein	3	5	260	10,450	480	46
193	Jersey	3	5	255	9,295	462	49
191	Jersey	3	5	254	8,184	733	89
195	Jersey	3	4	250	11,180	1,055	94
193	Jersey	3	4	248	9,078	565	62
193	Jersey	3	5	225	7,880	485	61
556	Holstein	3	5	215	10,405	463	44
569	Holstein	2	0	201	7,370	489	66
326	Ayrshire	12	1	190	9,415	364	38
569	Holstein	2	0	186	6,730	353	32
569	Holstein	2	0	179	6,826	390	57
326	Ayrshire	12	0	169	7,368	268	35
339	Ayrshire	4	3	162	7,470	442	59
156	Jersey	7	11	161	9,335	411	44
125	Jersey	12	3	159	11,590	656	56
275	Holstein	11	10	153	11,050	400	36
197	Jersey	3	0	135	7,587	308	40
340	Ayrshire	3	8	135	6,200	229	36
341	Ayrshire	3	5	133	12,890	495	38
154	Jersey	8	9	118	7,850	81	10
177	Jersey	5	7	114	7,430	195	26
520	Holstein	6	10	107	13,860	45	3
154	Jersey	8	7	99	12,310	76	6
128	Jersey	12	0	98	6,520	12	2
151	Jersey	9	1	73	12,750	51	4
266	Holstein	12	10	57	7,095	58	8
509	Holstein	8	4	34	8,842	81	9
521	Holstein	7	0	Open	10,118	0	0

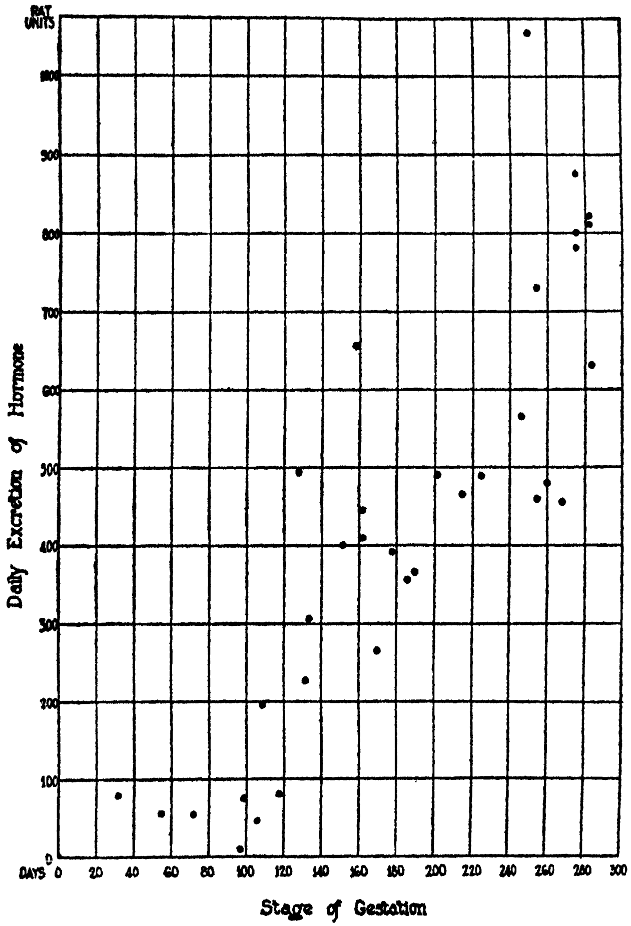


Fig. 3. The Variation in the Average Daily Excretion of the Ovarian Hormone as Extracted from Cow's Urine in All Stages of Gestation

It is seen that non-pregnant cows not in oestrum do not excrete the ovarian hormone; however, following conception there was a slight excretion of the ovarian hormone during the first 100 days. From 100 days until parturition there was a considerable increase in the daily rate of excretion amounting to 3.5 to 4 rat units daily.

The average of 12 cows from which urine was collected for forty-eight-hours indicate that although the cows show a large variation in many instances from day to day the average variation of all the cows is negligible. As to a comparison of day and night collections (8 a.m. to 8 p.m. and 8 p.m. to 8 a.m.) an average of 26 cows in all stages of gestation showed that there were 1117 cc., or 13 per cent more urine, and 29 rat units or 6 per cent more ovarian hormone excreted during the night collections. This preliminary work indicates that the volume of urine does not seem to influence the number of rat units excreted daily. In other words, the hormone excreted appears to be independent of the volume of urine.

TABLE 3

Results of alcoholic fractionation of oil extracts

COW NUMBER	AVERAGE NUMBER OF COLLECTIONS	STAGE GESTATION	POTENCY OF OIL EXTRACTS	POTENCY OF ALCOHOLIC EXTRACTS	ALCOHOLIC FRACTIONATION INCREASE	PERCENTAGE INCREASE
		days	*rat units per cc.*	*rat units per cc.*	*rat units*	
193	3	270	8.7	18.6	9.9	113
190	2	268	4.4	8.9	4.5	102
557	3	260	4.8	11.6	6.8	140
193	2	255	5.5	18.3	12.8	233
195	1	250	11.1	16.6	5.5	49
569	5	201	3.9	7.6	3.7	98
326	5	169	3.1	3.7	0.6	22
156	2	161	4.1	8.0	3.9	115
340	2	135	2.3	8.3	6.0	266
154	1	99	1.0	2.0	1.0	100
266	1	57	0.6	2.1	1.5	250
509	1	34	0.7	2.5	1.8	257
521	4	Open	0	0		

This preliminary work indicates that a twenty-four-hour sample of urine from which the ovarian hormone has been extracted with oil is a satisfactory method for measuring the ovarian hormone content of urine with the advancing stage of pregnancy. Therefore, using this method urine from cows in various stages of gestation was analyzed for its ovarian hormone content. Table 2 shows the results. It will be noted that there were included in the group of cows non-pregnant animals and others in all stages of gestation up to 281 days. Beginning with cows not in oestrum, it was found that no ovarian hormone was excreted.

During the first 100 days of pregnancy only a slight amount of the hormone is excreted. After approximately 100 days the daily excretion begins to increase. As shown in figure 3 there is considerable variation in the increase for individual animals; however, the average rate of increase is approximately 3.5 to 4 rat units daily up until parturition.

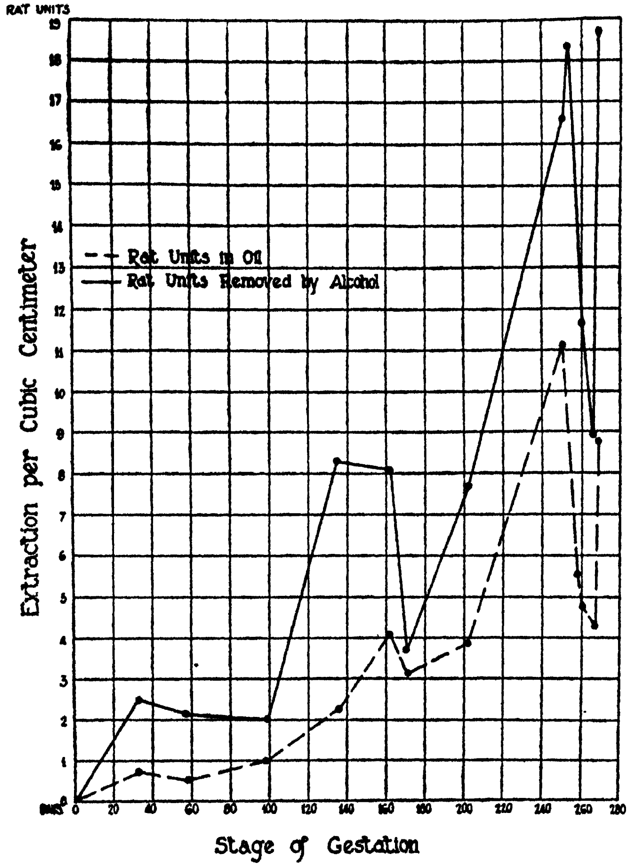


FIG. 4. RAT UNITS REMOVED BY ALCOHOLIC FRACTIONATION OF THE OIL CONTAINING THE OVARIAN HORMONE

Oils obtained from urine of cows at all stages of gestation were used for the alcoholic fractionation. The broken line shows the ovarian hormone present in the oil before extraction, the solid line after extraction with alcohol.

In addition to the presence of the oestrous producing hormone in the urine of pregnant cows, our data indicate the possible presence of a second factor which may inhibit the usual reaction of the ovarian hormone.

A number of samples of oil containing a known amount of the ovarian hormone obtained by the previous described method were fractionated with 95 per cent ethyl alcohol. The method employed in this fractionation process was as follows: The oil containing the ovarian hormone was well shaken with about ten times its volume of alcohol, the mixture was then allowed to separate, the alcohol was then removed and evaporated to a small volume. The residue was then taken up with a small amount of oil and the evaporation completed. This oil when retested gave a greater number of rat units per cubic centimeter than was in the original oil as is shown in table 3. It will be noted that there is an increase due to alcoholic fractionation from 22 to 266 per cent or an average increase of practically 125 per cent. Figure 4 shows the average increase for cows in the various stages of gestation.

DISCUSSION OF RESULTS

From the time of puberty there are obvious changes taking place in the mammary glands of the female, whereas in the male no further development ordinarily occurs. The source of the stimulation which produces such profound changes in the female mammary gland especially during pregnancy has long been sought. The earlier theories referred the source of the stimuli to reflexes originating in the nervous system. The experimental work of that period was chiefly concerned with the possibilities of normal growth of the mammary gland taking place if severed from the usual nervous connections. In recent years with the development of the hormone theory, the work has shifted to a study of the source of hormones which may activate the mammary glands.

Lane-Claypon and Starling (19) were the first to apply experimental methods to the study of the problem. From their work with extracts of the fetus and the placenta, the theory was advanced that the fetus was the source of the hormone which stimulates the growth of the mammary gland during pregnancy. Later others suggested the corpus luteum hormone and the so-called follicular or ovarian hormone as the source of the stimulus.

With the isolation and the perfection of a test of the ovarian or oestrous producing hormone, it became possible for the first time to experimentally determine the part played by the hormone in stimulating the growth of the mammary gland and the initiation of milk secretion.

As already suggested there are two modes of approach to the problem. It is possible to inject the purified hormone into experimental animals and note the effect produced in the gland or to determine under normal conditions of pregnancy the possible presence of the hormone in the circulation and in the urine.

From the anatomical studies of Hammond (16) who observed the most pronounced development of the mammary glands in first calf heifers during the latter months of gestation it might be expected that the greatest concentration of the hormone would be found at the same time. The data presented above are in accord with these observations and is believed to definitely prove for the first time that the oestrous producing hormone is actually present in the blood during pregnancy in increasing amounts which might be sufficient to produce the changes observed.

If the oestrous producing hormone is in part at least responsible for the growth of the mammary gland, it would be expected that the development of a large mammary gland would require a greater effective concentration of hormone than would the development of a small gland. Stated in other words, one cause of variation in the size and degree of development of the mammary glands may be due to the variation in the effective concentration of the hormone in various animals during pregnancy. Here again the data are in agreement with expectation for considerable variation in the excretion of the hormone at the same stage of gestation was observed. However, further observations will be necessary before the variations can be definitely proven to be due to genetic differences.

In addition to the presence of the oestrous producing hormone in the urine of pregnant cows, our data indicate the possible presence of a second factor which may inhibit the usual reaction of the ovarian hormone.

In explanation of these results it is postulated that there is present in the urine and absorbed by the oil some agent which has an inhibitory effect on the action of the oestrous producing hormone. When the original oil is tested, the presence of this factor reduces the apparent potency or hormone concentration. Upon separation or fractionation of the hormone from the original oil, this inhibiting agent is either not removed or is removed to a less extent than is the ovarian hormone.

It is well known that the vaginal smears of pregnant animals indicate an anoestrous condition. This phenomena is difficult to explain unless it is assumed that some inhibitory agent or hormone is present preventing the oestrous condition even when large amounts of the oestrous producing hormone are being excreted by such animals.

SUMMARY

1. A simple efficient method of extracting the ovarian hormone from the urine of pregnant cows by the use of oil is described.

2. As a result of the study of the diurnal variation in the volume of urine and its ovarian hormone content on a twelve-hour basis, it was found that the excretion of urine from 8 p.m. to 8 a.m. exceeded the collection during the day by 1117 cc. or 13 per cent; however, the difference in the excretion of the ovarian hormone was insignificant. For successive twenty-four-hour collections, little variation either in volume of urine or number of rat units was observed.

3. The yield of ovarian hormone is generally independent of the volume of urine excreted during a twenty-four-hour period.

4. Beginning with non-pregnant cows which excrete the ovarian hormone only at oestrum it was shown that following conception there was a slight excretion of the ovarian hormone during the first one hundred days. From one hundred days until parturition there was a considerable increase in the rate of excretion of the ovarian hormone, amounting to 3.5 to 4 rat units per day.

5. Following the alcoholic extraction of oils containing the ovarian hormone, it was found that there was an apparent increase in the number of rat units present in a definite volume of oil. These results are taken to indicate that there is present in the urine, and absorbed by the oil, some agent which has an inhibitory effect on the ovarian hormone. A tentative theory is advanced that this hormone or factor is responsible for maintaining the anoestrous type of cells in the vagina of pregnant animals.

REFERENCES

(1) Allen, E. 1922 The oestrous cycle in the mouse. Amer. Jour. Anat., xxx, 297–346.

(2) Allen, E., Colgate, C. E., Francis, B. F., Johnston, C. G., Robertson, L. S., Doisy, E. A., Gibson, H. V., and Kountz, W. B. 1924–1925 The hormone of the ovarian follicle; its localization and action in test animals, and additional points bearing upon the internal secretion of the ovary. Amer. Jour. Anat., xxxiv, 133–181.

(3) Allen, E., and Doisy, E. A. 1923 An ovarian hormone. Jour. Amer. Med. Assoc., lxxxi, 819–821.

(4) Allen, E., and Doisy, E. A. 1924 Induction of a sexually mature condition in immature animals. Amer. Jour. Physiol., lxix, 577–588.

(5) Allen, E., and Doisy, E. A. 1927 Ovarian and placental hormones. Physiol. Reviews, vii, 600–650.

(6) Allen, E., Pratt, J. P., and Doisy, E. A. 1925 The ovarian follicular hormone, its distribution in human genital tissues. Jour. Amer. Med. Assoc., lxxxv, 399–404.

(7) Bugbee, E. P., and Simond, A. E. 1926 Standardization of preparations of ovarian follicular hormone. Endocrinology, x, 191–200.

(8) Coward, K. H., and Burns, J. H. 1927 The variation in the unit of the oestrous-producing hormone. Jour. Physiol., lxii, 270–279.

(9) Doisy, E. A., Ralls, J. O., Allen, E., and Johnston, C. G. 1924 The extraction and some properties of an ovarian hormone. Jour. Biol. Chem., lxi, 711–727.

(10) Evans, H. M., and Burr, C. O. 1926 Increased efficiency of subcutaneous when compared with intraperitoneal administration of the ovarian hormone. Amer. Jour. Physiol., lxxvii, 518–521.

(11) Fels, E. 1926 Untersuchungen über das Ovarialhormon in Blute Gravider und nicht Gravider. Klin. Wchnschr., v, 2349–2352.

(12) Frank, R. T., Frank, M. L., Gustavson, R. G., and Weyerts, W. W. 1925 Demonstration of female sex hormone in circulating blood. Jour. Amer. Med. Assoc., lxxxv, 510.

(13) Frank, R. T., and Gustavson, R. G. 1925 The female sex hormone and the gestational gland. Jour. Amer. Med. Assoc., lxxxiv, 1715–1720.

(14) Frank, R. T., Gustavson, R. G., Halloway, J. H., Hydman, D., Krueger, H., and White, J. 1926 The occurrence and present chemical status of the female sex hormone. Endocrinology, x, 260–272.

(15) Halban, J. 1900 Ueber den Einfluss der Ovarian auf die Entwicklung des Genitales. Monatsscher f. Geburtsch. U. Gynak., xii, 496–506.

(16) Hammond, J. 1927 The Physiology of Reproduction in the Cow. Ed. I. Univ. Press Cambridge.

(17) Hisaw, F. L., and Meyer, R. K. 1929 The oestrous producing hormone in the urine of pregnant cows. Proc. Soc. Exper. Biol. and Med., xxvi, 586–588.

(18) Knauer, E. 1900 Die Ovarian Transplantation. Arch. f. Gynak., lx, 322–376.

(19) Lane-Claypon, J. E., and Starling, E. H. 1906 An experimental enquiry into the factors which determine the growth and activity of the mammary glands. Proc. Roy. Soc., lxxvii, Series B, 505–522.

(20) Loewe, S. 1926 Zur Pharmakologie des Ovarialhormons. Klinische Wochenschrift, v, 576.

(21) Long, J. A., and Evans, H. M. 1922 The oestrous cycle in the rat. Memoirs Univ. California, vi, 5–137.

(22) Papanicolaou, G. N., and Stockard, C. R. 1917 The existence of a typical oestrous cycle in the guinea pig with a study of its histological and physiological changes. Amer. Jour. Anat., xii, 225–265.

(23) Parkes, A. S. 1928 The physiology of ovarian activity. Biol. Rev. and Biol. Proc. of the Cambridge Philosophical Soc., iii, 206–252.

(24) Parkes, A. S., and Bellerby, C. W. 1926 Studies on the internal secretion of the ovary. The Effects of injection of the oestrous-producing hormone during pregnancy. Jour. Physiol., lxii, 145–155.

(25) Veler, C. D., and Doisy, E. A. 1928 Extraction of ovarian hormone from urine. Proc. Soc. Exp. Biol. and Med., xxv, 806–808.

(26) Zondek, B., and Ascheim, S. 1925 Experimentelle Untershungen über die funktion das Hormon des Ovari. Arch. f. Gynak., cxxvii, 256–292.

PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The annual meeting of the American Dairy Science Association was held in the new National Museum Building, Washington, D. C., on June 26 to 28, 1929. It was the third summer meeting with the program as a symposium of new completed research and activities, or reports of work in progress. The program was arranged by H. P. Davis of Nebraska, H. A. Ruehe of Illinois, and C. H. Eckles of Minnesota.

Members of the Bureau of Dairy Industry under the chairmanship of L. A. Rogers arranged special tours and entertainment of educational and historic interest for the ladies. The entire Association visited the Beltsville Farm on the afternoon of June 27th to inspect the studies in dairy cattle breeding, feeding, and management conducted by the Bureau of Dairy Industry. The following afternoon tours were made to Arlington and Mount Vernon. The banquet held at the Cosmos Club on the evening of the 27th, with H. A. Ruehe as toastmaster, was followed by short addresses by President G. C. White of Connecticut, by L. A. Rogers of Washington, D. C., and by F. B. Morrison of New York who gave an interesting illustrated address on his observations in Germany as a member of the Agricultural commission.

The executive committee meeting, attended by White (presiding), Ruehe, Sherman, Dahlberg, and Allen, considered several items of general interest. It was believed that the meeting place should be decided and announced a year in advance so that members can plan their work and vacations with a knowledge of the time of the next convention. The next meeting place will be Ames, Iowa and the committee tentatively accepted the invitation of the Pacific Coast members to hold the 1931 meeting in California, subject to later consideration for final decision. The committee decided that some effort should be made to further extend the field of the Journal and requested Dahlberg to plan for such extension.

President White appointed the following committee on nominations:

A. A. Boland (chairman)
C. H. Eckles
H. B. Ellenberger
H. W. Gregory
C. L. Roadhouse

This committee gave the following report:

President	J. M. Sherman H. P. Davis
Vice-President	H. C. Jackson C. R. Gearhart
Secretary-Treasurer	Fordyce Ely R. R. Graves
Editor	A. C. Dahlberg

The various sections which elected officers chose the following men:

Dairy Production	H. B. Ellenberger, chairman C. W. Turner, secretary
Dairy Manufacturers	C. L. Roadhouse, chairman C. D. Dahle, secretary

The committee on resolutions gave several resolutions which were quickly adopted and one, which after lengthy discussion, was tabled. Mention should be made of reports on the committees on chemical and on bacteriological analysis of milk and its products. The following resolutions of the committee on score cards was adopted:

> We, your committee on Legal Standards and Score cards, recommend that the score card be used as a guide in milk scoring contests grade milk as perfect when it complies in composition with the requirements of the state in which the contest is held.

The following program was presented.

GENERAL SESSION

June 26

President G. C. White, presiding

Address of Welcome. A. F. Woods, Director of Research, U. S. Department of Agriculture.

Address of the President. G. C. White, Connecticut Agricultural College.

Announcements.

The Bureau of Dairy Industry. O. E. Eeed, Bureau of Dairy Industry, U. S. Department of Agriculture.

Economic Aspects of Diseases of Dairy Cattle. J. R. Mohler, Bureau of Animal Industry, U. S. Department of Agriculture.

Present Trends of Business and Economic Development of the Dairy Industry. Roy C. Potts, Bureau of Agricultural Economics, U. S. Department of Agriculture.

Some Phases of the Phosphorus Deficiency Problem in the Nutrition of Dairy Cattle. C. H. Eckles, University of Minnesota.

Effect of Feeding Flourine Upon the Health and Teeth of Dairy Cattle. George E. Taylor, Michigan State College.

The Nervous Reaction of Animals on a Deficiency Diet (Film). J. S. Hughes and H. W. Cave, Kansas State Agricultural College.

Discussion.

Buffet Lunch in Laboratories of Bureau of Dairy Industry.

Nutritional Value of Raw and Pasteurized Milks. J. M. Brannon and M. J. Prucha, University of Illinois.

The Taste of Market Milk as Influenced by Certain of its Constituents. C. L. Roadhouse, University of California.

Metals and Milk. E. S. Guthrie, Cornell University, and C. L. Roadhouse, University of California.

Buffer Action of Milk:

- a. Factors that Determine the Buffer Action of Milk. E. O. Whittier, Bureau of Dairy Industry, U. S. Department of Agriculture.
- b. Variations in the Buffer Value of Herd Milk. Paul D. Watson, Bureau of Dairy Industry, U. S. Department of Agriculture.
- c. Buffer Value of Proprietary Milks and Milks from Different Individuals. George E. Holm, Bureau of Dairy Industry, U. S. Department of Agriculture.

Value of Photography in Dairy Cattle Breeding and Type Investigations. M. H. Fohrman, Bureau of Dairy Industry, U. S. Department of Agriculture.

Excretion of Ovarian Hormones during Pregnancy. C. W. Turner and C. W. Nibler, University of Missouri.

Philadelphia Price Plan. I. Ralph Zollers, Inter-State Milk Producers Association.

Relationships of Temperature to Physical, Chemical and Biological Changes in Milk. J. C. Marquardt, New York Agricultural Experiment Station.

College Creameries. H. A. Ruehe, University of Illinois.
Discussion.
Meeting for Organization of Dairy Economic Section.
Receptions to Members. Hotel Harrington.

DAIRY MANUFACTURERS—BACTERIOLOGY—MARKET MILK

June 27

W. H. Martin, presiding

Estimation of Fat and Phospholipins in Buttermilk. L. M. Thurston, University of West Virginia.
Fat Determination in the Evaporated Milk Industry. Frank S. Rice, Evaporated Milk Association.
Some Chemical Changes in Frozen Milk Occurring in Storage. E. O. Anderson, Connecticut Agricultural College.
Effect of Heat Treatment on the Stability or Viscosity of Cultured Buttermilk. R. W. Bell, Bureau of Dairy Industry, U. S. Department of Agriculture.
A Study of Washed Curd Cheese. W. V. Price and Walter Hochstrasser, University of Wisconsin.
Relation of Cheese Composition to Quality. J. L. Sammis and Leo Germain, University of Wisconsin.
Some Observations on Process Cheese. H. L. Templeton and H. H. Sommer, University of Wisconsin.
Rennet Coagulation of Milk from Individual Cows. F. L. Schacht and H. H. Sommer, University of Wisconsin.
A Formula Expressing the Relation between Fat and Protein in Milk. A. E. Perkins, Ohio Agricultural Experiment Station.
Comparison of Methylene Blue, Janus Green and Plate Counts in Grading Raw Milk. M. J. Prucha, University of Illinois.
The Foaming of Milk. C. S. Leete, Bureau of Dairy Industry, U. S. Department of Agriculture.
Some New Facts Concerning Ropy Milk. C. N. Stark and Pauline Stark, Cornell University.
The Bacterial Proteolytic Enzymes. Their Activity of Different pH values and the Effect of Sodium Chloride. E. H. Parfitt and George Spitzer, Purdue University.
Committee Reports and Election of Officers of Manufacturing Section.
Inspection of Beltsville Farm and Experimental Work in Dairy Cattle Breeding, Nutrition and Management.
Banquet—Cosmos Club.

OFFICIAL TESTING AND DAIRY PRODUCTION

June 27

J. R. Dice, presiding

A Statistical Study of the Reliability of Bi-Monthly Tests. Warren Gifford, University of Missouri.

Advantages of Bi-Monthly Testing. Lynn Copeland, American Jersey Cattle Club.

Studies of the Bi-Monthly Test. J. C. McDowell, Bureau of Dairy Industry, U. S. Deparment of Agiculture.

Discussion.

R. B. Becker, presiding

The Influence of Low Fat Ration on Yield of Milk and Fat. L. A. Maynard and C. M. McCay, Cornell University.

Various Methods of Comparing Transmitting Ability of Dairy Sires by Daughter and Dam Comparison. C. W. Turner, University of Missouri.

Roughage Grinding. Thomas Olson, South Dakota State College

Digestibility of the Total Ration as Affected by Ground Versus Unground Roughage. Paul S. Williams, Pennsylvania State College.

Pasture Fertilization. C. H. Parson, Synthetic Nitrogen Products Corporation.

Yield and Composition of Pasture Grass. H. B. Ellenberger, University of Vermont.

Significance and Causes of Daily Fluctuation in Weight in Dairy Cattle. C. L. Allen and R. G. Maxwell, Cornell University.

Wintering Dairy Heifers by Means of the Self Feeder. H. O. Henderson, University of West Virginia.

Committee Reports and Election of Officers of Production Section.

Inpection of Beltsville Farm and Experimental Work in Dairy Cattle Breeding, Nutrition and Management.

Banquet—Cosmos Club.

DAIRY EXTENSION

June 28

G. A. Williams, presiding

The Relation of the Bureau of Dairy Industry to the Office of Coöperative Extension Work. C. B. Smith, Office of Coöperative Extension Work, U. S. Department of Agriculture.

Supervision of Dairy Herd Improvement Associations. C. R. Gearhart, Pennsylvania State College.

Future Development of Dairy Herd Improvement Association. A. J. Cramer, University of Wisconsin.

District Conference for Cow Testers. Floyd Johnson, Iowa State College.

Coöperative Creameries as a Means for Promoting Herd Testing. H. R. Searles, University of Minnesota.

Scoring Type and Pedigree as a Stimulus to Proving the Herd Sire. E. J. Perry, New Jersey College of Agriculture.

Scoring Dairy Sires and Their Progeny. M. J. Regan, University of Missouri.

Bull Associations in Pennsylvania. S. J. Brownell, Pennsylvania State College.

Development and Promotion of 4 H Calf Club Activities. R. A. Turner, Office of Coöperative Extension Work, U. S. Department of Agriculture.

Timely Dairy Information and Subject Matter for Dairy Extension. A. C. Baltzer, Michigan State College.

Some Results of Recent Tabulations of Dairy Herd Improvement Association Records. J. C. McDowell, Bureau of Dairy Industry, U. S. Department of Agriculture.

Use of Local Leaders in Work of Dairy Herd Improvement Association. J. H. Brock, University of Illinois.

Committee Reports and Election of Officers of Extension Section.

DAIRY MANUFACTURERS—BACTERIOLOGY—MARKET MILK

June 28

W H. Martin, presiding

Keeping Quality and Shrinkage of Print Butter in Storage. William White, Bureau of Dairy Industry, U. S. Department of Agriculture.

Quantitative Changes in the Microflora of Butter. H. Macy, University of Minnesota.

Sandiness in Nut Ice Cream. W. H. E. Reid, University of Missouri.

Use of Honey in Ice Cream Manufacture. P. H. Tracy, University of Illinois.

Utilization of Fruits in Frozen Dairy Product. H. J. Mack, Massachusetts State College.

Relationship of the Amount and Quality of Gelatin to the Viscosity and Plasticity of the Ice Cream Mix. Alan Leighton and Floyd E. Kurtz, Bureau of Dairy Industry, U. S. Department of Agriculture.

Study of the Effect of Aging Ice Cream Mixes. J. C. Hening, New York Agriculture Experiment Station.

Effect of Dried Skimmilk on the Texture and Flavor of Ice Cream. O. E. Williams and S. E. Hall, Bureau of Dairy Industry, U. S. Department of Agriculture.

Effect of Homogenization on Protein Stability. E. J. Doan, Pennsylvania State College.

Factors that Control the Color of Evaporated Milk. B. V. Webb and George E. Holm, Bureau of Dairy Industry, U. S. Department of Agriculture.

Evaporated Milk and Its Place in the Diet. Frank S. Rice, Evaporated Milk Association.

Committee Reports.

Mt. Vernon and Arlington Tours.

INDEX TO VOLUME XII

ALFALFA plant. I. The calcium, phosphorus, and nitrogen metabolism of dairy cattle, The physiological effect of rations restricted principally or solely to the.................... 445

American Dairy Science Associaciation, Proceedings of the annual meeting of the............. 507

ANDEREGG, L. T., and HAMMER, B. W. Proteolysis by *Streptococcus lactis*........................... 114

BABCOCK and the Mojonnier methods for butterfat in ice cream, A comparison of the modified.... 473

Bacteria in milk, The comparative accuracy of the direct microscopic and agar plate methods in determining numbers of......... 304

Bacterial count of ice cream held at freezing temperatures, The. 182

Bacteriological methods for condensed, evaporated, and dry milk of the American Dairy Science Association, Progress report of sub-committee on..... 374

(Bacteriology) A study of the proteolytic action of specific organisms and groups of organisms in butter made from graded cream......................... 1

(Bacteriology) Proteolysis by *Streptococcus lactis*. 114

Baer, Arthur Christopher, 1884–1929...................... 191

BARTLETT, J. W., and BENDER, C. B. A study of the factors affecting the growth of dairy heifers. Part II........................ 37

BECKER, R. B., and McGILLIARD, P. C. A suggested simplification of the present system of official testing with the dairy breeds of cattle 337

BENDER, C. B. (See Bartlett, J. W.)........................... 37

BENTON, ANNE G. Variations in the milk from different quarters of the same udder; their significance in studies of coagulability. 481

Book Review 419

BRANDT, P. M. (See Haag, J. R.).. 445

Breeds of cattle, A suggested simplification of the present system of official testing with the dairy......... 337

BREW, JAMES D. The comparative accuracy of the direct microscopic and agar plate methods in determining numbers of bacteria in milk 304

Bromthymol blue milk test and the methylene blue reduction test for determining quality of milk, A comparison of the 367

BUCHANAN, J. H., and LOWMAN, O. E. Seasonal variations in the freezing point of milk....... 484

Butter made from graded cream, A study of the proteolytic action of specific organisms and groups of organisms in.................. 1

Butter, The mold and yeast count as an index of the keeping quality of........... 351

Butterfat in ice cream, A comparison of the modified Babcock and the Mojonnier methods for...... 473

Butterfat percentage and milk yield, Effect of heat (oestrum) on................... 464

BUTTON, FORREST C. (See Mueller, William S.)................ 320

BUTTON, FORREST C. (See Russell, Walter C.)............ 231

CALCIUM phosphate in fresh milk. The solubility of...... 405
Calves, Further studies on the rôle of vitamin C in the nutrition of... 394
CARODEMOS, P. P. (See Miscall, J.) 379
Cattle, A suggested simplification of the present system of official testing with the dairy breeds of . 337
Cattle, Effect of cottonseed meal and linseed oil meal on the consistency of feces of dairy........ 410
Cattle, The physiological effect of rations restricted principally or solely to the alfalfa plant I. The calcium, phosphorus, and nitrogen metabolism of dairy... 445
CAULFIELD, W. J. (See Martin, W. H.)........................ 193
CAVANAUGH, G.W. (See Miscall J.) 379
CHASE, E. S., and KING, F. G. A comparison of the modified Babcock and the Mojonnier methods for butter fat in ice cream...... 473
Cheese, A color defect of process. 377
Cheese, The relation of hydrogen-ion concentration to the texture of Emmenthal or Swiss......... 289
Citric acid and sodium citrate in starter cultures, The use of..... 21
Coagulability, Variations in the milk from different quarters of the same udder; their significance in studies of.............. 481
Color defect of process cheese, A. 377
COPELAND, LYNN. Effect of heat (oestrum) on butterfat percentage and milk yield............. 464
Copper in dairy products and its solution under varying conditions. II....................... 379
Copper in powdered whole milk, The nutritional value of........ 90
CORDES, W. A. (See Hunziker, O. F.)......................140, 252
Corrosion caused by washing powders, chemical sterilizers, and refrigerating brines, Metals in dairy equipment............. 252
Corrosion in milk products and its effect on flavor, Metals in dairy equipment..................... 140
Cottonseed meal and linseed oil meal on the consistency of feces of dairy cattle, Effect of........ 410
Cow's urine, The ovarian hormone content of pregnant............ 491
Cream, A study of the proteolytic action of specific organisms and groups of organisms in butter made from graded............. 1
Cream mixtures when homogenized, Some factors affecting the fat clumping produced in milk and.......................... 211

DAHLBERG, A. C. (See Hening, J. C.)................... 129
Dairy products and its solution under varying conditions. II. Copper in....... 379
DEVEREUX, E. D. A comparison of the bromthymol blue milk test and the methylene blue reduction test for determining quality of milk................ 367
DEYSHER, E. F., WEBB, B. H., and HOLM, GEORGE E. The relations of temperature and time of forewarming of milk to the heat stability of its evaporated product....................... 80
DOAN, F. J. Some factors affecting the fat clumping produced in milk and cream mixtures when homogenized.................. 211
DOWNS, P. A. Progress report of sub-committee on bacteriological methods for condensed, evaporated, and dry milk of the American Dairy Science Association.................... 374

ECKLES, C. H. (See Thurston, L. M.) 394
Egg products in the manufacture of ice cream, The use of dehydrated 320
Egg yolk in ice cream, The use of... 193
Equipment, Corrosion caused by washing powders, chemical sterilizers, and refrigerating brines, Metals in dairy 252
Equipment, Metallic corrosion in milk products and its effect on flavor, Metals in dairy 140
Escherichia coli and *Serratia marcescens* to survive 62.8°C., for thirty minutes in milk, The ability of 202
Evaporated product, The relations of temperature and time of forewarming of milk to the heat stability of its 80

FARRALL, A. W. The application of steam for heating and sterilizing dairy equipment..... 95
Fat clumping produced in milk and cream mixtures when homogenized, Some factors affecting the. 211
Fat production of Guernsey cattle, The relation between weight and 60
Feces of dairy cattle, Effect of cottonseed meal and linseed oil meal on the consistency of...... 410
Flavor, Metals in dairy equipment. Metallic corrosion in milk products and its effect on.. 140
Forewarming of milk to the heat stability of its evaporated product, The relations of temperature and time of 80
FOUTS, E. L. A study of the effectiveness of sodium hypochlorite in sterilizing creamery equipment 51
Freezing point of milk, Seasonal variations in the 484

GERDEMAN, ALICE E. (See Weinzirl, John) 182
Growth of dairy heifers, Part II, A study of the factors affecting the 37
Guernsey Cattle, The relation between weight and fat production of 60

HAAG, J. R., JONES, J. S., JONES, I. R., and BRANDT, P. M. The physiological effect of rations restricted principally or solely to the alfalfa plant. I. The calcium, phosphorus, and nitrogen metabolism of dairy cattle. 445
HAMMER, B. W. (See Anderegg, L. T.) 114
HASTINGS, E. G. Book review.... 419
Heat (oestrum) on butterfat percentage and milk yield, Effect of 464
Heat stability of its evaporated product, The relations of temperature and time of forewarming of milk to the 80
Heifers. Part II, A study of the factors affecting the growth of dairy 37
HENING, J. C., and DAHLBERG, A. C. The effects of certain salts on the physical properties of ice cream mixes 129
HERRINGTON, B. L. (See Whitaker, Randall) 456
HOLM, GEORGE E. (See Deysher, E. F.) 80
Homogenized, Some factors affecting the fat clumping produced in milk and cream mixtures when.. 211
Hormone content of pregnant cow's urine, The ovarian 491
HUFFMAN, C. F., and MOORE, L. A. Effect of cottonseed meal and linseed oil meal on the consistency of feces of dairy cattle..... 410
HUGHES, J. S. (See Titus, R. W.). 90

Hunziker, O. F., Cordes, W. A., and Nissen, B. H. Metals in dairy equipment. Metallic corrosion in milk products and its effect on flavor................ 140
Hunziker, O. F., Cordes, W. A., and Nissen, B. H. Metals in dairy equipment. Corrosion caused by washing powders, chemical sterilizers, and refrigerating brines................. 252
Hydrogen-ion concentration to the texture of Emmenthal or Swiss cheese, The relation of the...... 289
Hypochlorite in sterilizing creamery equipment, A study of the effectiveness of sodium.......... 51

ICE cream, A comparison of the modified Babcock and the Mojonnier methods for butterfat in........................ 473
Ice cream held at freezing temperatures, The bacterial count of............................ 182
Ice cream mixes, The effects of certain salts on the physical properties of.................. 129
Ice cream mix to its basic viscosity, A device for reducing an.. 285
Ice cream, the antirachitic value of irradiated. 231
Ice cream, The use of egg yolk in... 193
Ice cream, The use of dehydrated egg products in the manufacture of........................... 320

JONES, J. R. (See Haag, J. R.)......................... 445

KAHLENBERG, Orme J. (See Russell, Walter C.)........... 231
King, F. G. (See Chase, E. S.).. 473
Koestler, G. A. (See Roadhouse, C. L.).................. 421
Krauss, W. E. Studies on the nutritive value of milk. I. The production of nutritional anemia in albino rats through exclusive whole milk diets................ 74
Krauss, W. E. Studies on the nutritive value of milk.......... 242
Krauss, W. E. Studies on the nutritive value of milk. III. The supplementary value of various constituents of synthetic basal rations...................... 438

LINSEED oil meal on the consistency of feces of dairy cattle, Effect of cottonseed meal and... 410
Lowman, O. E. (See Buchanan, J. H.)......................... 484

MACY, H., and Richie, H. B. The mold and yeast count as an index of the keeping quality of butter....................... 351
Martin, W. H., and Caufield, W. J. The use of egg yolk in ice cream........................ 193
McGilliard, P. C. (See Becker, R. B.)............ 337
Metals in dairy equipment, Metallic corrosion in milk products and its effect on flavor.......... 140
Metals in dairy equipment, Corrosion caused by washing powders, chemical sterilizers and refrigerating brines............. 252
Methylene blue reduction test for determining quality of milk, A comparison 'of the bromthymol blue milk test and the.......... 367
Milk, A comparison of the bromthymol blue milk test and the methylene blue reduction test for determining quality of...... 367
Milk, an unappreciated but important factor in the pasteurization of........................ 385
Milk and cream mixtures when homogenized, Some factors affecting the fat clumping produced in...................... 211

Milk from different quarters of the same udder; their significance in studies of coagulability, Variations in the.................... 481
Milk of the American Dairy Science Association, Progress report of sub-committee on bacteriological methods for condensed, evaporated and dry..... 374
Milk, Studies on the nutritive value of..... 242
Milk, The comparative accuracy of the direct microscopic and agar plate methods in determining numbers of bacteria in 304
Milk, The nutritional value of copper in powdered whole....... 90
Milk. I. The production of nutritional anemia in albino rats through exclusive whole milk diets, Studies on the nutritive value of... 74
Milk, The solubility of calcium phosphate in fresh. 405
Milk. III. The supplementary value of various constituents of synthetic basal rations, Studies on the nutritive value of........ 438
Milk taste, Contribution to the knowledge of................. . 421
Milk to the heat stability of its evaporated product, The relations of temperature and time of forewarming of... 80
Milk, Seasonal variations in the freezing point of....... 484
Milk yield, Effect of heat (oestrum) on butterfat percentage and.... 464
Miscall, J., Cavanaugh, G. W., and Carodemos, P. P. Copper in dairy products and its solution under varying conditions. II.... 379
Mojonnier milk tester for the routine determination of vanillin with preliminary discussion of the towt lead number, The use of the........................ 469
Mojonnier methods for butterfat in ice cream, A comparison of the modified Babcock and the....... 473
Mold and yeast count as an index of the keeping quality of butter.. 351
Moore, L. A. (See Huffman, C. F.)........... 410
Mueller, William S., and Button, Forrect C. The use of dehydrated egg products in the manufacture of ice cream....... 320

Nibler, C. W., and Turner, Chas. W. The ovarian Hormone content of pregnant cow's urine 491
Nissen, B. H. (See Hunziker, O. F.)........... ....140, 252
Nutrition of calves, Further studies on the rôle of vitamin C in the......................... 394
(Nutrition) The antirachitic value of irradiated ice cream.......... 231
Nutritional value of copper in powdered whole milk, The...... 90
Nutritive value of milk. I. The production of nutritional anemia in albino rats through exclusive whole milk diets, Studies on the.......... 74
Nutritive value of milk, Studies on the. 242
Nutritive value of milk. III. The supplementary value of various constituents of synthetic basal rations, Studies on the. .. 438

Ovarian Hormonec ontent of pregnant cow's urine, The... 491

Palmer, L. S. (See Thurston, L. M.)...................... 394
Parfitt, E. H. (See Spitzer, George)...................... 1
Pasteurization of milk, An unappreciated but important factor in the.................. 385

(Pasteurization) The ability of *Escherichia coli* and *Serratia marcescens* to survive 62.8°C., for thirty minutes in milk...... 202
Price, Walter V. A color defect of process cheese............... 377
Proteolysis by *Streptococcus lactis*. 114
Proteolytic action of specific organisms and groups of organisms in butter made from graded cream, A study of............. 1

QUALITY of butter, The mold and yeast count as an index of the keeping.................... 351
Quality of milk, A comparison of the bromthymol blue milk test and the methylene blue reduction test for determining........ 367

RATIONS restricted principally or solely to the alfalfa plant. I. The calcium, phosphorus, and nitrogen metabolism of dairy cattle, The physiological effect of............................ 445
Richie, H. B. (See Macy, H.)... 351
Roadhouse, C. L., and Koestler, G. A. Contribution to the knowledge of milk taste......... 421
Russell, Walter C., Button, Forrest C., and Kahlenberg, Orme J. The antirachitic value of irradiated ice cream.......... 231

SALTS on the physical properties of ice cream mixes, The effects of certain 129
Seasonal variations in the freezing point of milk.................. 484
Sherman, J. M., Stark, C. N., and Stark, Pauline. An unappreciated but important factor in the pasteurization of milk...... 385
Sommer, H. H. (See Templeton, Hugh L.)...................... 21
Spitzer, George, and Parfitt, E. H. A study of the proteolytic action of specific organisms and groups of organisms in butter made from graded cream........ 1
Stark, C. N. (See Sherman, J. M.)............................ 385
Stark, Pauline. (See Sherman, J. M.)......................... 385
Starter cultures, The use of citric acid and sodium citrate in...... 21
Steam for heating and sterilizing dairy equipment, The application of......................... 95
Sterilizing creamery equipment, A study of the effectiveness of sodium hypochlorite in......... 51
Sterilizing dairy equipment, The application of steam for heating and.......................... 95
Streptococcus lactis, Proteolysis by. 114
Swiss Cheese, The relation of the hydrogen-ion concentration to the texture of Emmentahl or... 289

TANNER, FRED W., and WINDSOR, MARY F. The ability of *Escherichia coli* and *Serratia marcescens* to survive 62.8°C., for thirty minutes in milk...... 202
Templeton, Hugh L., and Sommer, H. H. The use of citric acid and sodium citrate in starter cultures................. 21
Testing with the dairy breeds of cattle, A suggested simplification of the present system of official........................ 337
Thurston, L. M., Palmer, L. S., and Eckles, C. H. Further studies on the rôle of vitamin C in the nutrition of calves....... 394
Titus, R. W., and Hughes, J. S. The nutritional value of copper in powdered whole milk......... 90
Towt, L. V. The use of the Mojonnier milk tester for the routine determination of vanillin with preliminary discussion of the Towt lead number.......... 469

Turner, Charles W. The relation between weight and fat production of Guernsey cattle...... 60
Turner, Chas. W. (See Nibler, C. W.)........................ 491

Vanillin with preliminary discussion of the Towt lead number, The use of the Mojonnier milk tester for the routine determination of.................. 469
Viscosimeter, A method for determination of absolute viscosity by means of the torsion pendulum.......................... 456
Viscosity, A device for reducing an ice cream mix to its basic....... 285
Vitamin C in the nutrition of calves, Further studies on the rôle of.......................... 394

Watson, **Paul** D. The relation of the hydrogen-ion concentration to the texture of Emmentahl or Swiss cheese..... 289
Webb, B. H. (See Deysher, E. F.).......................... 80
Weight and fat production of Guernsey cattle, The relation between...... 60
Weinzirl, John, and **Gerdeman, Alice** E. The bacterial count in ice cream held at freezing temperatures.................. 182
Whitaker, Randall. A device for reducing an ice cream mix to its basic viscosity............... 285
Whitaker, Randall, and **Herrington**, B. L. A method for determination of absolute viscosity by means of the torsion pendulum viscosimeter...... ... 456
Whittier, E. O. The solubility of calcium phosphate in fresh milk.......................... 405
Windsor, Mary F. (See Tanner, Fred W.)...................... 202

Yeast count as an index of the keeping quality of butter, The mold and...................... 351

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or before ....

Return Date	Return Date